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Within 30 min, acute pyridostigmine treatment induced elevations of serum growth hormone levels to a greater extent in Flinders Sensitive Line (FSL) and Sprague-Dawley (SD) rats compared to Flinders Resistant (FRL) rats, without affecting temperature or activity. This finding confirms the cholinergic supersensitivity of the FSL rats but also indicates that the FRL rats are very resistant as well. All groups exhibited growth hormone elevations at 30 min following 12 mg/kg, so this dose of pyridostigmine was given chronically for 14 days before challenging the rats with chlorpyrifos (CPF, 60 mg/kg orally) or diisopropylfluorophosphate (DFP, 1 mg/kg sc). Hypothermia induced by CPF or DFP was greater over the first two hr in the FSL rats, as expected. However, hypothermia developed more rapidly in pyridostigmine-pretreated rats, suggesting that pyridostigmine does not protect against the centrally mediated effects of these two anticholinesterase agents. Pyridostigmine did reduce the diarrhea induced by DFP, indicating that it can protect against peripherally mediated effects. It is concluded that pyridostigmine is not a very effective prophylactic against centrally acting anticholinesterase agents.

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David Oberstreet 7-25-98

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Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity

INTRODUCTION

In the assessment of risk to individuals exposed to known or potential toxicological agents. there needs to be a consideration of the possibility that especially sensitive populations exist. For example, some individuals have reported side effects after taking pyridostigmine to protect them against potential nerve gas exposure and others have not. Other individuals have reported increased sensitivity to a variety of chemical agents, usually after a triggering exposure to a specific chemical such as an organophosphate (OP) pesticide (e.g., Miller and Mitzel, 1995). The hypothesis that a genetically based cholinergic supersensitivity might underlie the increased sensitivity of these vulnerable human populations will be addressed in the present communication by describing in detail the features of an animal model with cholinergic supersensitivity which is also more sensitive to a variety of drugs and other chemical agents and which may, therefore, mimic the human condition labeled Multiple Chemical Sensitivity (MCS). In the body of this paper results on the effects of acute pyridostigmine on serum growth hormone levels in this animal model will be presented. In addition, the (in)ability of chronic pyridostigmine to protect these animals agains the effects of OP will be presented and discussed.

Multiple Chemical Sensitivity

Multiple Chemical Sensitivity (MCS) is a syndrome in which, following acute or repeated exposure to one or more chemicals, most commonly organophosphate pesticides (OPs), individuals become overly sensitive to a wide variety of chemically-unrelated compounds. These can include ethanol, caffeine and other psychotropic drugs (Ashford and Miller, 1989, 1991; Bell et al., 1992; Cullen, 1987; Miller, 1994). The symptoms of MCS often reported include fatigue, cognitive difficulties, depression, irritability, headaches, dyspnea, digestive problems, musculoskeletal pain, and numbness in their extremities. These conditions often overlap those of common medical illnesses such as depression, somatization disorder, chronic fatigue syndrome, fibromyalgia, asthma and others. However, a distinguishing feature of MCS is the strong belief of the patients that their symptoms are brought on by common exposures to low levels of volatile organic chemicals such as fragrances, insecticides, traffic exhaust, disinfectants and perfumes.

An important observation in this field is that MCS patients usually report that other individuals simultaneously exposed to similar amounts of pesticides, e.g., family members, friends, or co-workers, did not develop MCS or even experience transient illness. This observation suggests that a subset or subsets of the people may be more vulnerable to developing MCS. Indeed, some (Black et al., 1990; Simon et al., 1990), but not all (Fiedler et al., 1992) researchers have reported greater rates of depression and somatization disorder predating the "initiating" chemical exposure among persons with MCS as compared to controls. Thus, any model must take into account why only some individuals develop MCS after exposures to pesticides or other chemicals.

The FSL Rat Model

One such model which will be described in the subsequent sections of this paper is the FSL (Flinders Sensitive Line) rat. This rat was developed by selective breeding for increased sensitivity to an OP, so it shares some etiologic similarity to patients with MCS who were exposed to pesticides. The FSL rat model is one with which we have had extensive experience, particularly in research on depressive syndromes (Overstreet, 1993; Overstreet and Janowsky, 1991; Overstreet et al., 1995). Analogies between depressed states and MCS, as well as substance hypersensitivities in FSL rats, first brought our attention to the potential value of this model for experimental studies of MCS, as recently described (Overstreet et al., 1996). Further, because the FSL rats were selectively bred for increased responses to the organophosphate, DFP, it is possible that they may have some special relevance to Gulf War Illness, commonly reported in individuals exposed to the carbamate, pyridostigmine.

Selective Breeding for OP Differences. The FSL rat model arose from a selective breeding program designed to produce two lines of rats, one with high (FSL) and one with low (Flinders Resistant Line - FRL) sensitivity to the anticholinesterase agent, diisopropylfluorophosphate (DFP) (Overstreet et al., 1979; Russell et al., 1982). The selective breeding program, which was initiated at Flinders University in Adelaide, Australia, utilized three somatic measures of DFP (Overstreet et al., 1979; Russell et al., 1982). A rank-order system was used to give equal weighting to each of the three variables. Rats which had the lowest average ranks were intermated to establish and maintain the line of more sensitive rats (FSL), while rats which had the highest average ranks were intermated to establish and maintain the line of more resistant rats (FRL). Subsequent studies showed that randomly bred Sprague-Dawley rats, from which the lines were originally derived, were not different from the FRL rats. On the other hand, FSL rats were significantly more sensitive to DFP than the other two groups (Overstreet et al., 1979; Russell et al., 1982).

Biochemical Mechanisms. This project was initiated, in part, to develop genetically resistant lines of rats so that the biochemical mechanisms of resistance could be compared with those of tolerance. Early studies ruled out changes in acetylcholinesterase as a mechanism to account for the differential sensitivity of FSL and FRL rats to DFP (Overstreet et al., 1979; Russell and Overstreet, 1987; Sihotang and Overstreet, 1983), just as has been found for tolerance development (See Russell and Overstreet, 1987). Because DFP-tolerant rats were subsensitive to the effects of muscarinic agonists (e.g., Overstreet et al., 1972, 1973, 1974), the effects of muscarinic agonists on the FSL and FRL rats were examined (Overstreet 1986;

Overstreet and Russell, 1982; Overstreet et al., 1986a,b). These studies showed that the FSL rats were more sensitive to pilocarpine, arecoline and oxotremorine than were the FRL rats; this supersensitivity was seen for a variety of responses, including hypothermia, reduced locomotor activity, and suppression of bar-pressing for water reward (Overstreet and Russell, 1982). Thus, FSL rats, developed by selectively breeding for increased sensitivity to DFP, exhibited opposite changes in sensitivity to muscarinic agonists compared to DFP-tolerant rats.

Biochemical studies indicated that the FSL rats exhibited greater numbers of muscarinic receptor binding sites in the hippocampus and striatum than the FRL rats (Overstreet et al., 1984; Pepe et al., 1988)., but there were no differences in acetylcholine turnover (Overstreet et al., 1984). Thus, once again, the FSL rats appear to represent the converse of DFP-tolerant rats; having increased numbers of receptors rather than reduced numbers (See Russell and Overstreet, 1987). It appears that both tolerance and acute sensitivity to cholinergic agents is related to postsynaptic cholinergic mechanisms rather than presynaptic. Although in both instances, there have been detectable changes in the muscarinic receptors themselves, there are some findings, such as the increased sensitivity of FSL rats to noncholinergic agents (See Section below), which suggest that post-receptor mechanisms may also contribute.

<u>Behavioral Features of FSL Rats.</u> The FSL and FRL rats differ on a large number of behavioral tasks, as recently summarized in several review papers (Overstreet et al., 1995, 1996). In this section we will highlight a number of the key differences. The FSL rats have been reported to have lower locomotor activity than the FRL rats under a number of experimental conditions (Bushnell et al., 1995; Overstreet, 1986; Overstreet and Russell, 1982) but not all (Criswell et al., 1994; Rezvani et al., 1994). They are even less active when stressed prior to exposure to the open field (Overstreet, 1986; Overstreet et al., 1989a).

Results from several other behavioral paradigms are consistent with the view that depressive-like psychomotor retardation symptoms are more apparent in the FSL rats after exposure to stressors. For example, the FSL rats are impaired in active avoidance paradigms compared to the FRL rats (Overstreet and Measday, 1985; Overstreet et al., 1990a, 1992a). Another stress-oriented paradigm which has provided important information about behavioral differences between FSL and FRL rats is the forced swim test. Upon initial exposure in a cylinder (l8-20 cm diameter) of water (25 oC), FSL rats are more immobile than the FRL rats (Overstreet, 1986; Overstreet et al., 1986a, Pucilowski and Overstreet, 1993; Schiller et al., 1992; Zangen et al., 1997). This exaggerated immobility of the FSL rats is counteracted by chronic but not acute treatment with antidepressants (Overstreet, 1993; Pucilowski and Overstreet, 1993; Schiller et al., 1992; Zangen et al., 1997). These findings provide further support for the contention that the FSL rat is a useful animal model of depression.

There are also differences in reward-related behaviors between the FSL and FRL rats which are consistent with the proposal that the FSL rats are a model of depression. In operant bar-pressing tasks, the FSL rats bar-pressed at lower rates and had to be maintained at a lower percentage of their free-feeding body weight and have smaller food pellets (37 vs. 45 mg) in order to keep their motivation sufficiently high to complete the session (Bushnell et al., 1995; Overstreet and Russell, 1982). Despite these differences in reward-related and stress-related behaviors, there appear to be no differences between the FSL and FRL rats in the ability to perform a matching-to-sample task (Bushnell et al., 1995). However, this test was carried out under normal, unstressed conditions, and it is not clear whether similar findings would obtain under stressed conditions. For example, FSL and FRL rats have similar amounts of saccharin consumption under baseline conditions, but the FSL rats exhibit greater decreases after exposure to chronic mild stress (Pucilowski et al., 1993).

The FSL rats also have elevated REM sleep and reduced latency to REM sleep (Shiromani et al., 1988, Benca et al., 1996), as has been reported in human depressives (Benca et al., 1992) Human depressives are also more sensitive to the effects of cholinergic agonists on REM sleep latency (Janowsky et al., 1994), but there are no data in the FSL rats regarding drug effects yet.

In sum, the FSL rats and depressed humans exhibit a large number of behavioral and physiological similarities (See Overstreet, 1993; Overstreet et al., 1995, 1996, for details).

Multiple Chemical Sensitivity in FSL Rats. Clinical observations suggest that MCS may be initiated by acute or chronic exposure to a variety of chemical agents (Miller and Mitzel, 1995). Because the FSL rats were selectively bred to have increased responses to the anticholinesterase agent, DFP, it should not be surprising that they exhibited increased sensitivity to muscarinic agonists (Daws et al., 1991; Overstreet, 1986; Overstreet and Russell, 1982; Overstreet et al., 1992a,b; Schiller et al., 1988). It has also been reported that human depressives are also more sensitive to directly acting muscarinic agonists (Gann et al., 1992; Gillin et al., 1991) as well as anticholinesterases (Gann et al., 1992; Janowsky and Overstreet, 1995; Nurnberger et al., 1989; O'Keane et al., 1992; Schreiber et al., 1992; Sitaram et al., 1987). A similar increased sensitivity to anticholinesterases has been observed in MCS patients (Cone and Sult, 1992; Miller and Mitzel, 1995; Rosenthal and Cameron, 1991) but there are no published data for MCS patients regarding sensitivity to direct cholinergic agonists. FSL rats are also more sensitive to nicotine, which interacts with nicotinic cholinergic receptors (Schiller and Overstreet, 1993).

The cholinergic system interacts with many other major neurotransmitter systems, including serotonergic, dopaminergic, GABAergic, and noradrenergic. Having animals with clear-cut differences in the cholinergic system afforded us the opportunity to test how the FSL and FRL rats differ in response to drugs interacting with these other neurotransmitter systems. Evidence from various drug challenge studies, in which relatively selective drugs are given to FSL and FRL rats, have revealed a substantial number of differences between the FSL and FRL rats, as summarized in Table 1. FSL rats were found to exhibit a greater degree of hypothermia after a variety of drugs which interact with the serotonin 5-HT1A receptor (Wallis et al., 1988; Overstreet et al., 1992a, 1994). This outcome is consistent with much of the evidence suggesting supersensitive serotonergic mechanisms in depressives (Arango et al., 1990; Arora and Meltzer, 1989; Mikuni et al., 1991), but is not consistent with neuroendocrine studies reporting blunted responses to serotonergic agonists, which suggests serotonergic hyposensitivity (Lesch et al., 1990; Meltzer and Lowy, 1987). There are no data on the effects of selective serotonergic agents in MCS patients, but there is one report of supersensitive responses in individuals with chronic fatigue syndrome, which is related to MCS (Backheit et al., 1992).

To date no evidence has been obtained to indicate any differences in responses to noradrenergic agents in the FSL rats (Overstreet, 1989; Overstreet et al, 1989a). In contrast, there are quite a number of differences with regard to dopaminergic agents (Table 1). The FSL rats are supersensitive to the hypothermic (Crocker and Overstreet, 1991) and aggression-promoting (Pucilowski et al., 1991) effects of apomorphine, a mixed D1/D2 agonist, and quinpirole, a selective D2 agonist. On the other hand, the FSL rats were subsensitive to the stereotypy-inducing effects of similar doses of the same compounds and there were no apparent differences in dopamine D2 receptors between FSL and FRL rats (Crocker and Overstreet, 1991).

These opposite changes in sensitivity in the various functions might be related to the type of modulation of these functions by the cholinergic and dopaminergic systems. Stimulation of both cholinergic and dopaminergic systems promotes hypothermic and aggressive responses (Cox et al., 1980; Pucilowski, 1987; Ray et al., 1989), but cholinergic stimulation reduces activity and stereotypy, thereby opposing the effects of dopaminergic stimulation (Fibiger et al., 1970; Klemm, 1989).

The FSL and FRL rats are differentially sensitive to the effects of several pharmacological agents which have modulatory roles at the GABA-A receptor, as summarized in Table 1. However, as with the case of dopamine agonists, the differential effects are observed only for some actions of the drugs, not for all. For example, the hypothermic effects of ethanol are significant higher in the FSL rats compared to the FRL rats, but the sedative effects are similar (Overstreet et al., 1990b). Similarly, the behavioral suppressant effects of diazepam are significantly greater in the FSL rats (Pepe et al., 1988), but its anxiolytic effects in the two lines are comparable (Schiller et al., 1991). The fact that these two commonly abused psychotropic drugs both modulate GABA function at the GABA-A receptor suggests that there might be differences in GABA-A receptor subtype composition between the two lines, but there is not biochemical evidence for such differences as yet. Furthermore, despite differences in sensitivity to the hypothermic effects of ethanol, the FSL and FRL rats do not differ in their rates of voluntary ethanol consumption (Overstreet et al., 1992a).

In summary, it appears that the FSL rat is more sensitive to a variety of chemical agents in addition to the OP anticholinesterase agent for which they were selectively bred. In this regard, the FSL rat is somewhat analogous to MCS patients who have become more sensitive to a range of agents following exposure to OP anticholinesterases. The extent of the similarity between the

FSL rats and MCS patients, on one hand, and human depressives and MCS patients, on the other, has been more extensively evaluated in recent reviews (Overstreet et al., 1996, 1997a).

Acute Effects of Pyridostigmine

Pyridostigmine bromide is a quaternary carbamate anticholinesterase agent which has been used routinely in the treatment of myasthenia gravis (Taylor, 1996). It was prescribed to Persian Gulf War participants as a prophylactic against the possible exposure to nerve agents (Keeler et al., 1991). A subset of these individuals have reported very various problems, but it is not yet clear whether the problems are related to their exposure to pyridostigmine, to other agents during the Gulf War, or to stress. The present proposal addresses the hypothesis that the individuals developing these problems may have had a genetic cholinergic supersensitivity, undetectable under normal conditions, which made them more sensitive to pyridostigmine and/or other agents to which they were exposed. Because the FSL and FRL rats were genetically selected to respond differently to cholinergic agonists, they are ideal animals to test this hypothesis. It was predicted that the cholinergically supersensitive FSL rats would be more sensitive to the effects of pyridostigmine than the FRL rats or an outbred Sprague-Dawley strain of rats. The serum levels of growth hormone were selected as one variable to assess because there is evidence that pyridostigmine produces abnormal elevations of this hormone in several human populations with abnormalities (Chaudhury et al., 1997; Ghigo et al., 1993; Lucey et al., 1993; O'Keane et al., 1992, 1994). Telemetrically monitored core body temperature and general activity were selected as additional variables which could be measured reliably without influencing growth hormone levels and which might also be affected by pyridostigmine.

Chronic Effects of Pyridostigmine.

Pyridostigmine was given chronically to troops which had been assigned to duty in the Gulf War in the hope that it would protect them against the consequences of possible exposure to nerve agents which, like pyridostigmine, inhibit cholinesterases. Because it had been widely used in the treatment of myasthenia gravis (Taylor, 1996) without incident and had been given chronically to a group of human volunteers maintained under high ambient temperatures (Wenger et al., 1993), it was assumed that pyridostigmine itself would not have any negative consequences. However, whether it could act as a prophylactic against the effects of nerve agents had not been adequately tested at the time this proposal was submitted, although there had been reports of its usefulness in conjunction with other prophylactics such as oximes and anticholinergics (e.g., Koplovitz and Stewart, 1994).

The basic thesis of our proposal is that there will be individual differences in sensitivity to pyridostigmine and to nerve agents and that, consequently, pyridostigmine may not be able to protect all individuals from the effects of exposure to nerve agents. To address this question, it was proposed to use both sexes of the FSL and FRL rats, differentially sensitive to cholinergic agents, and SD rats which had been chronically treated with saline or pyridostigmine for two weeks. The animals would then be challenged with the commonly used pesticide, chlorpyrifos (CPF), or the commonly used experimental anticholinesterase agent, diisopropylfluorophosphate (DFP), and core temperature and activity recorded.

BODY

Methods

Animals. The FSL and FRL rats were selected from breeding colonies maintained at the University of North Carolina at Chapel Hill and randomly bred Sprague-Dawley (SD) rats (from which the FSL and FRL rats were originally derived) were obtained from Harlan Sprague-Dawley

(Indianapolis, IN) to act as a reference group. Both males and females were used. The SD rats were included in the research design in order to determine whether both FSL and FRL rats are different from normal. Until surgery they were maintained in groups of 3-5 in polypropylene cages under conditions of constant temperature and humidity and a reversed light:dark cycle (lights off from 1000-2200).

Surgery. Recording of locomotor activity and core body temperature in freely moving rats was accomplished by the implantation of a transmitter weighing 7.0 g (Model TA-11ETA-F40-L20). This transmitter had temperature- and motion-sensitive elements and when actuated by passing a magnet along the rat's abdomen, transmitted information to a computer where it was stored using Data Quest IV software (Data Sciences, Inc., St. Paul, MN).

At about 70 days of age the rats were injected i.p. with sodium pentobarbital (35 mg/kg) to induce anesthesia for implanting the telemetry transmitters, which provided continuous monitoring of core body temperature and general activity. The fur over the ventral abdominal area was clipped and a 3-cm longitudinal incision was made along the midline about 1 cm below the sternum. The radiotransmitter was inserted into the abdominal cavity and sutured to the peritoneal wall with 4-0 silk thread. After testing the transmitter with an AM receiver, the skin was closed. The rats were placed in single polypropylene cages after surgery and were closely monitored until they were active.

Acute Pyridostigmine Procedures. After a one week period to allow full recovery (Rezvani et al., 1994), the FSL, FRL and SD rats were adapted to the home cages for at least 24 hr and then injected s.c. with a mixture of peripherally acting methyl atropine (MA, 2.0 mg/kg) and oxotremorine (OXO, 0.2 mg/kg) to determine hypothermic responses. This treatment was given to insure that each group of rats were either sensitive (FSL) or resistant (FRL) to a well characterized cholinergic agonist. This information is necessary to interpret the hypothermic responses to pyridostigmine.

Approximately three days after the MA/OXO challenge, the rats were given pyridostigmine (PYR) bromide by gavage. The design called for four groups (vehicle and 4, 12, 36 mg/kg), with ten rats per group. The animals were run in squads of 10 rats, the capacity of the computer, in a counterbalanced order. The average temperatures and general activity counts recorded during the hour preceding the gavage and those recorded at approximately 30 min after the injection were used in statistical analyses.

The rats were sacrificed by decapitation exactly 30 min after the oral administration of pyridostigmine, any signs of diarrhea were noted, and blood was collected into centrifuge tubes. The tubes were centrifuged and the plasma was collected and stored at -20 °C for later determination of growth hormone levels, using a kit obtained by NIDDK.

Chronic Pyridostigmine Procedures. The intermediate dose of 12 mg/kg pyridostigmine was selected as the chronic dose; it was gavaged in a volume of 3 ml/kg and the controls received an equivalent volume of isotonic saline orally. Some variations of the above procedures were necessary due to the requirement of a 14-day chronic treatment period with pyridostigmine or saline. Chronic treatment was initiated approximately 1-2 days prior to surgery to implant the transmitter. Surgery was performed in the afternoon, approximately 4-6 hr after the daily treatment with pyridostigmine and proceeded without incident. After a one week period of recovery, the rats were placed on the receivers for the monitoring of temperature and activity baselines for at least 48 hr prior to the challenge with CPF or DFP.

Exactly 30 min after the 14th treatment with pyridostigmine, the rats received one of three challenge treatments: Saline (3 ml/kg orally), CPF (60 mg/kg in 3 ml/kg orally), or DFP (1 mg/kg intramuscularly). Temperature and activity were monitored for the next two hr and then the rats were sacrificed by decapitation. The blood was stored for the later determination of cholinesterase activity and possible growth hormone levels and the brains were stored for the later determination of cholinesterase activity and muscarinic receptor binding. Only the physiological data will be communicated in this report. The assays for the biochemical measures have not been completed as yet and the data will be commnicated in the subsequent final report in July, 1999.

There was also a variation in the timing of the MA/OXO challenge as the experiments progressed. Initially, the challenge was conducted two days prior to the start of the chronic treatment phase, using a Physiotemp telethermometer and temperature probe. After the recording of baseline temperatures, the rats were given sc injections of the MA/OXO (2/0.2 mg/kg) mixture and core body temperatures were recorded at 30, 60 and 90 min after the injections. These recordings provided information about the sensitivity of the cholinergic system in the various groups prior to the start of chronic pyridostigmine or saline treatment.

When it became apparent that pyridostigmine was altering the hypothermic sensitivity to CPF, the timing of the MA/OXO challenge was changed to nine days after the initiation of the chronic treatment period (approximately one week after the implantation of the transmitters). Temperature was now monitored telemetrically and 48 hr of baseline and the complete time course of oxotremorine-induced hypothermia were recorded. This procedure was adopted so that the potential effects of chronic pyridostigmine on a cholinergic drug which is not dependent upon cholinesterase inhibition for its effects. Oxotremorine interacts directly with central cholinergic receptors (since its peripheral effects were blocked by MA) to induce its hypothermic effects.

Results and Discussion

Acute Pyridostigmine. The effects of acute pyridostigmine or saline treatment on activity and temperature were described in last year's annual report. Table 2 from that report is included here to emphasize the lack of strain and gender differences in the changes in temperature produced by either compound (See Table 2). For the 4 mg/kg dose only, the female rats exhibited larger increases in temperature than their male counterparts. The serum growth hormone assays from these animals have now been completed and are included in this report (Fig. 1).

The values for growth hormone levels at 30 min after the administration of pyridostigmine are illustrated in Figure 1. The data were initially analyzed for gender differences and, since none were found, the results for males and females were combined for each line. In each line, pyridostigmine produced an elevation at the intermediate doses, with 12 mg/kg being significantly higher than saline in each line. However, both the FSL and the SD rats exhibited greater elevations of growth hormone than the FRL rats at the at 4 mg/kg dose of pyridostigmine, suggesting greater cholinergic sensitivity in these lines of rats.

When considered in conjunction with the negative data on temperature and activity presented in our previous annual report, these results suggest that the site(s) with which pyridostigmine interacts to induce increases in growth hormone may be located outside of the central nervous system or the blood-brain barrier. Irrespective of this conclusion, it is also clear that the FSL rats exhibited a greater growth hormone elevation to pyridostigmine than did the FRL rats. Such a finding would be consistent with the suggestion that the FSL rat may be a genetic animal model of depression, because depressed humans are also more sensitive to pyridostigmine-induced changes in growth hormone (O'Keane et al., 1992).

The SD rats appeared to be as sensitive to the effects as pyridostigmine on growth hormone as the FSL rats (Fig. 1), but were intermediate in their sensitivity to oxotremorne (Fig. 2). These SD rats were obtained from Harlan and there has been other evidence that this substrain of SD may be very different from another substrain of SD rats obtained from Holtzman. For example, it has been recently reported that the SD/Har rats are more sensitive to the hypothermic effects of 8-OH-DPAT, a selective serotonin1A (5-HT1A) receptor agonist, than are the SD/Hol rats (Barlcells-Olivera et al., 1997). We have also reported that the FSL rats are similarly more sensitive to the hypothermic effects of 8-OH-DPAT compared to the FRL rats (Overstreet et al., 1994). Because it is likely that the cholinergic and serotonergic systems interact (Overstreet et al., 1998; see Appendix), it is possible that the SD/Har rats may be more sensitive than the SD/Hol rats to cholinergic agonists as well as 5-HT1A receptor agonists. This hypothesis will be tested in the final year of the project by comparing the hypothermic responses after oxotremorine of both groups of SD rats with those of the FSL and FRL rats. If the findings come out as expected, questions about what is an appropriate reference population for the FSL and FRL rats will be further reinforced.

In conclusion, it should be stressed that the FSL rats exhibited a supersensitive growth hormone response to pyridostigmine compared to the FRL rats. Since there were no changes in temperature and activity in these same rats, these findings are evidence for the growth hormone changes probably being mediated by sites outside of the blood-brain barrier. Thus, the cholinergic supersensitivity exhibited by the FSL rats can be observed at sites outside of the brain. This conclusion is consistent with other recent findings in these rats indicating increased cholinergic sensitivity of the FSL rats in the small intestine (Djuric et al., 1995) and the upper airways (Djuric et al., in press).

Chronic Pyridostigmine. The results of the MA/OXO challenges on core body temperature prior to the beginning of chronic treatment are summarized in Figure 2. The FSL rats are clearly more sensitive to the hypothermic effects of MA/OXO and the FRL rats are resistant, both in reference to the FSL rats and the randomly bred SD rats. Note also that the female rats are more sensitive. These data, therefore, confirm the differences seen previously using telemetrically monitored temperature (Overstreet et al., 1997a,b; previous annual report)..

A wealth of physiological data has been collected in this project. Only the data on temperature will be summarized here. To illustrate the changes in temperature which occurred during the various treatments, we have decided to present the data as a series of graphs containing the last 4.5 hr prior to the last chronic treatment, the 30 min following this last chronic treatment and the 2 hr following the challenge treatment. Manipulations of these basic data have then been conducted to provide statistical analyses of the respective groups.

The first set of figures illustrate that a saline challenge in the rats chronically treated with pyridostigmine or saline produced relatively few effects, as might be expected (Fig. 3A-F). However, there are distinct trends in the data for the some of rats chronically treated with pyridostigmine to have elevated or reduced temperatures relative to the saline-treated controls (See Fig. 3). As a consequence of this finding, the effects of challenge drugs were expressed as changes from the 30-min temperatures just prior to their administration.

The second set of figures illustrate the hypothermic effects of CPF in rats chronically treated with pyridostigmine or saline. In five out of six of the groups the rats which had been chronically pretreated with pyridostigmine exhibited a more rapid decrease in temperature after CPFchallenge (See Figure 4).

As illustrated in Figure 5, DFP also produced a more rapid decrease in temperature in all of the rats chronically pretreated with pyridostigmine. These effects were quite striking even though the sample sizes of some of the groups were quite small (3-5 in the FSL groups).

As indicated in Figures 4 and 5, the groups chronically pretreated with pyridostigmine exhibited more rapid decreases in temperature than the groups chronically pretreated with saline. To evaluate these differences, the average temperatures of the various treatment groups at 1 and 2 hr after the acute challenge treatments of DFP and CPF (1.5 and 2.5 hr after the last pyridostigmine or saline treatment) were compiled in tabular form and analyzed by two-way Analysis of Variance, with strain and pretreatment as the two main factors. These findings are summarized in Tables 3 & 4. Pretreattment effects were significat at 1 but not a 2 hr, while strain differences were significant at both time points. In all cases, the FRL rats were the most resistant, while the FSL rats were the most sensitive to DFP but equally as sensitive to CPF as the SD rats.

The greater effects of both CPF and DFP in the pyridostigmine-pretreated rats could be explained by a pharmacokinetic mechanism. Because pyridostigmine is attached to cholinesterase molecules in the periphery, there are fewer binding sites for CPF and DFP. Therefore, more of these agents should penetrate the brain and one could therefore expect a more rapid rate of decline in core body temperature, as seen in Figs. 4 & 5. If this hypothesis is correct, then the brain cholinesterase activity may be lower in the rats which had been pretreated with pyridostigmine and acutely challenged with CPF or DFP. The results of these cholinesterase assays are not available yet, but will be communicated in the next report.

Another approach to the explanation for the differences in sensitivity in pyridostigmine- and saline-treated rats is to introduce a challenge to an agent whose effects are not dependent on cholinesterase inhibitor. As indicated in Figure 6, it appears that chronic pyridostigmine treatment also sensitized some animals to the hypothermic effects of OXO, a directly acting cholinergic agonist. However, the pattern of temperature changes is different for OXO than for CPF or DFP. For all three drugs the peak change in temperature was fairly similar for rats pretreated with either pyridostigmine or saline. For OXO, there was a prolonged hypothermia in the pyridostigmine-treated FSL groups (Fig. 6B & 6E), while it was shorter in the SD males (Fig. 6D). In contrast, for CPF and DFP, there was a more rapid decline in body temperature (Fig. 4 & 5).

Assessment of Diarrhea. Diarrhea is a frequent symptom in animals exposed to anticholinesterase agents and is probably a reasonable index of peripheral cholinergic overstimulation. Evidence of diarrhea was observed at the time of sacrifice two hr after administration of the CPF, DFP or saline challenges and 2.5 hr after the last treatment with pyridostigmine or saline. There were no signs of diarrhea in the rats challenged with saline, confirming the low incidence of diarrhea by this oral dose of pyridostigmine reported in our last annual report. CPF, which was orally administered at a intermediate dose of 60 mg/kg (Nostrand et al., 1997) also produced relatively little diarrhea, with only two FSL male rats (one pretreated with pyridostigmine, one pretreated with saline) showing signs of diarrhea. The incidence of diarrhea was higher following the sc administration of 1 mg/kg DFP. Across all groups, a total of 2 out of 40 rats pretreated with pyridostigmine showed signs of diarrhea after DFP challenge, while 11 out of 37 rats pretreated with saline were similarly affected (chi square = 7.41, $p < 0.05$). Almost all of the animals exhibiting diarrhea were either FSL or SD rats, suggesting increased peripheral cholinergic sensitivity in these groups, a conclusion which has been reinforced by other recent studies (Djuric et al., 1995; in press). These findings are also suggestive of the possibility that pyridostigmine may offer protection against a key peripheral cholinergic symptom induced by

DFP. Further groups of FSL and FRL rats will be tested after DFP administration this year and additional data on the incidence of diarrhea may allow an even more conclusive statement.

Table 1

Multiple Chemical Sensitivity in FSL Rats

Drug Classes to which FSL rats are more sensitive than FRL rats

Drug Class	Compound	Responses
Anticholinesterase	DFP	Temperature/drinking
Anticholinesterase	Physostigmine	Temperature/activity
Muscarinic Agonist	Oxotremorine	Temperature/activity
Muscarinic Agonist	Pilocarpine	Temperature/activity
Muscarinic Agonist	Arecoline	Temperature/activity
Nicotinic Agonist	Nicotine	Temperature/activity
Dopamine D1/2 Agonist	Apomorphine	Temperature
Dopamine D2 Agonist	Quinpirole	Temperature
Dopamine D2 Antagonist	Raclopride	Catalepsy
5-HT-1B Agonist	mCPP	Temperature/activity
5-HT-1A Agonist	8-OH-DPAT	Temperature
5-HT-1A Agonist	Buspirone	Temperature
Benzodiazepine Agonist	Diazepam	Temperature/activity
Multiple (GABA, 5-HT)	Ethanol	Temperature

Table 2

Change in Core Temperature after Oral Administration of

Saline or Pyridostigmine in FSL, FRL and SD Rats

Line/Sex	Dose of Pyridostigmine (mg/kg)			
	0.0	4.0	12.0	36.0
SD-male	+0.4±0.1	+0.6±0.1	+0.2±0.1	0.0±0.2
SD-female	+0.5±0.1	+1.0±0.1	+0.5±0.1	-0.2±0.2
FSL-male	+0.3±0.3	+0.3±0.2	+0.9±0.4	-0.2±0.2
FSL-female	+0.4±0.2	+0.7±0.1	+0.8±0.2	+0.1±0.2
FRL-male	+0.3±0.2	+0.2±0.1	+0.3±0.2	+0.1±0.2
FRL-female	+0.3±0.2	+0.4±0.2	+0.8±0.1	+0.2±0.1
One-Way ANOVA	0.50	5.17**	2.55	0.91

**Significant differences, $p < 0.01$

Table 3

Hypothermic Effects one hour after DFP or CPF Treatment in Rats Chronically Pretreated with Saline or Pyridistigmine

STRAIN/SEX	CPF		DFP	
	SAL	PYR	SAL	PYR
SD-F	36.90±0.18	36.07±0.08	37.27±0.25	36.95±0.09
FSL-F	37.13±0.11	36.47±0.09	36.29±0.34	35.07±0.19
FRL-F	37.85±0.04	37.53±0.04	37.87±0.11	37.32±0.07
SD-M	37.18±0.05	36.93±0.06	37.28±0.15	36.62±0.15
FSL-M	36.70±0.12	36.83±0.09	36.95±0.19	35.78±0.23
FRL-M	37.17±0.03	37.00±0.04	37.70±0.07	37.20±0.08
F (Treatment) =	14.52, $p < 0.01$		11.22, $p < 0.01$	
F (Strain/Sex)	81.47, $p < 0.001$		109.76, $p < 0.001$	

Table 4

Hypothermic Effects two hours after DFP or CPF Treatment in Rats Chronically Pretreated with Saline or Pyridistigmine

STRAIN/SEX	CPF		DFP	
	SAL	PYR	SAL	PYR
SD-F	35.20±0.08	35.34±0.06	35.43±0.09	36.10±0.09
FSL-F	36.07±0.07	35.64±0.06	34.50±0.11	34.53±0.06
FRL-F	37.41±0.10	37.47±0.06	37.26±0.06	37.16±0.05
SD-M	36.10±0.12	35.78±0.12	36.07+0.07	35.73±0.10
FSL-M	35.64±0.11	35.94±0.07	35.23±0.12	34.56±0.04
FRL-M	37.03±0.06	36.87±0.08	36.94±0.66	36.69±0.10
F(treatment) =	0.45, NS		0.75, NS	
F (strain/sex)	481.36, $p < 0.001$		667.5, $p < 0.001$	

Figure Captions

Figure 1. Dose-Dependent Effects of Pyridostigmine on Serum Growth Hormone Levels in FSL, FRL and SD Rats. Rats were treated with pyridostigmine or saline by gavage 30 min prior to sacrifice by decapitation. Blood was collected in heparinized tubes and stored frozen at -20 oC until assayed by a kit from NIDDK. Values represent the mean values for 9-15 rats. *Significantly different from saline treatment.

Figure 2. Strain and Gender-Dependent Effects of Oxotremorine in FSL, FRL and SD Rats. After the recording of baseline temperatures, rats were injected sc with a mixture of 2 mg/kg methyl atropine nitrate and 0.2 mg/kg oxotremorine sesquifumarate. The scores represent the mean decrease from baseline temperature (oC) for 25-30 rats at 60 min after the injection. Different letters indicate that the groups are significantly different from each other according to Newman-Keuls tests.

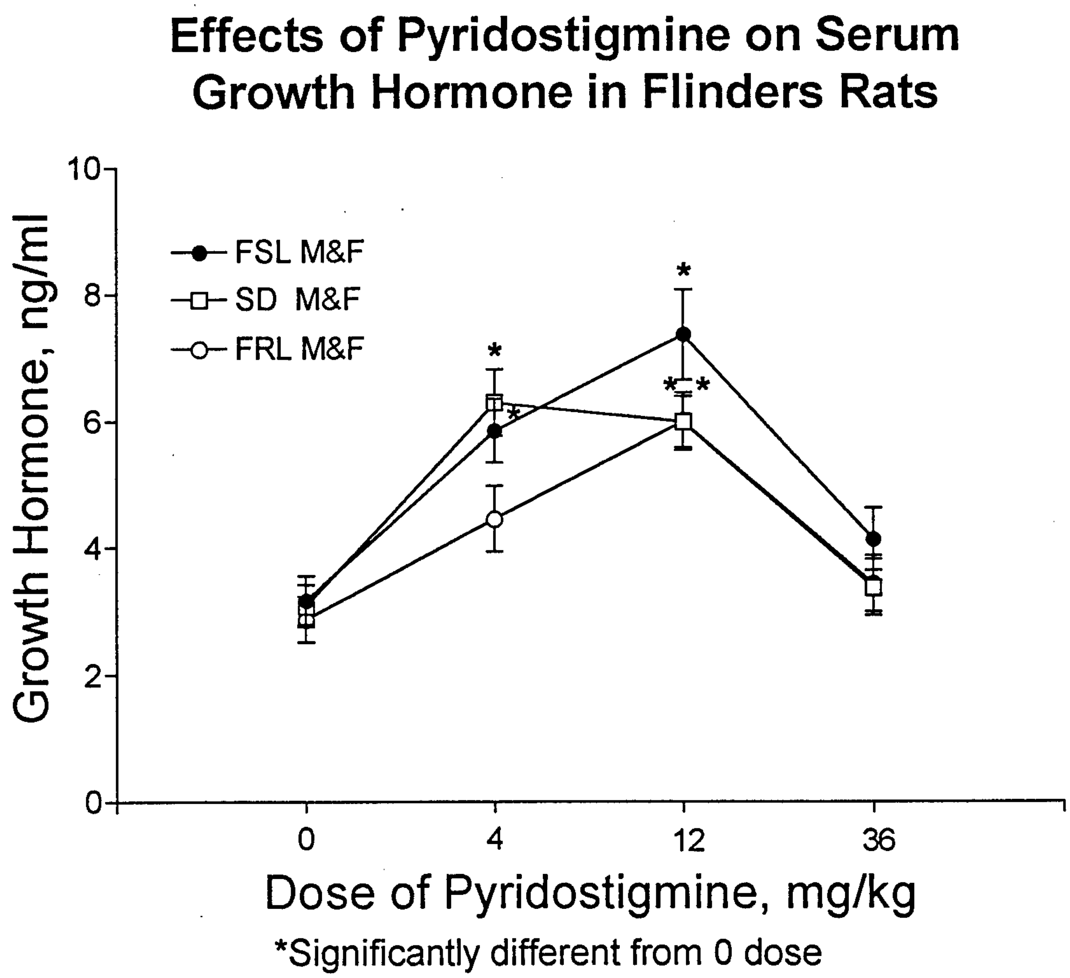
Figure 3A-F. Changes in Telemetrically Monitored Temperature in FSL, FRL and SD Rats following Chronic Saline or Pyridostigmine Treatment and Acute Saline Challenges. Rats were chronically treated by gavage with pyridostigmine (12 mg/kg) or saline for 14 days. The saline challenge (gavage or intramuscular) was given 30 min after the 14^{th} treatment and temperature was monitored for a further 2 hr. The data represent the mean temperatures for 8-10 animals. Standard errors are omitted for clarity.

Figure 4A-F. Changes in Telemetrically Monitored Temperature in FSL, FRL and SD Rats following Chronic Saline or Pyridostigmine Treatment and Acute Chlorpyrifos (CPF) Challenges. Rats were chronically treated by gavage with pyridostigmine (12 mg/kg) or saline for 14 days. The CPF challenge (60 mg/kg by gavage) was given 30 min after the 14^{th} treatment and temperature was monitored for a further 2 hr. The data represent the mean temperatures for 8-10 animals. Standard errors are omitted for clarity.

Figure 5A-F. Changes in Telemetrically Monitored Temperature in FSL, FRL and SD Rats following Chronic Saline or Pyridostigmine Treatment and Acute Diisopropylfluorophosphate (DFP) Challenges. Rats were chronically treated by gavage with pyridostigmine (12 mg/kg) or saline for 14 days. The DFP challenge (1 mg/kg, s.c.) was given 30 min after the 14th treatment and temperature was monitored for a further 2 hr. The data represent the mean temperatures for 8-10 animals. Standard errors are omitted for clarity.

Figure 6A-F. Changes in Telemetrically Monitored Temperature in FSL, FRL and SD Rats following Chronic Saline or Pyridostigmine Treatment and Acute Oxotremorine (OXO) Challenges. Rats were chronically treated by gavage with pyridostigmine (12 mg/kg) or saline for 10 days. The OXO challenge (0.2 mg/kg with 2 mg/kg methyl atropine, s.c.) was given 30 min after the 10th treatment and temperature was monitored for a further 6 hr. The data represent the mean temperatures for 4-5 animals. Standard errors are omitted for clarity.

Fig. 1



Effects of Pyridostigmine on Serum
Growth Hormone in Flinders Rats
FSL M&F
SD M&F
FRL M&F
Growth Hormone, ng/ml
Dose of Pyridostigmine, mg/kg
*Significantly different from 0 dose

Fig. 2

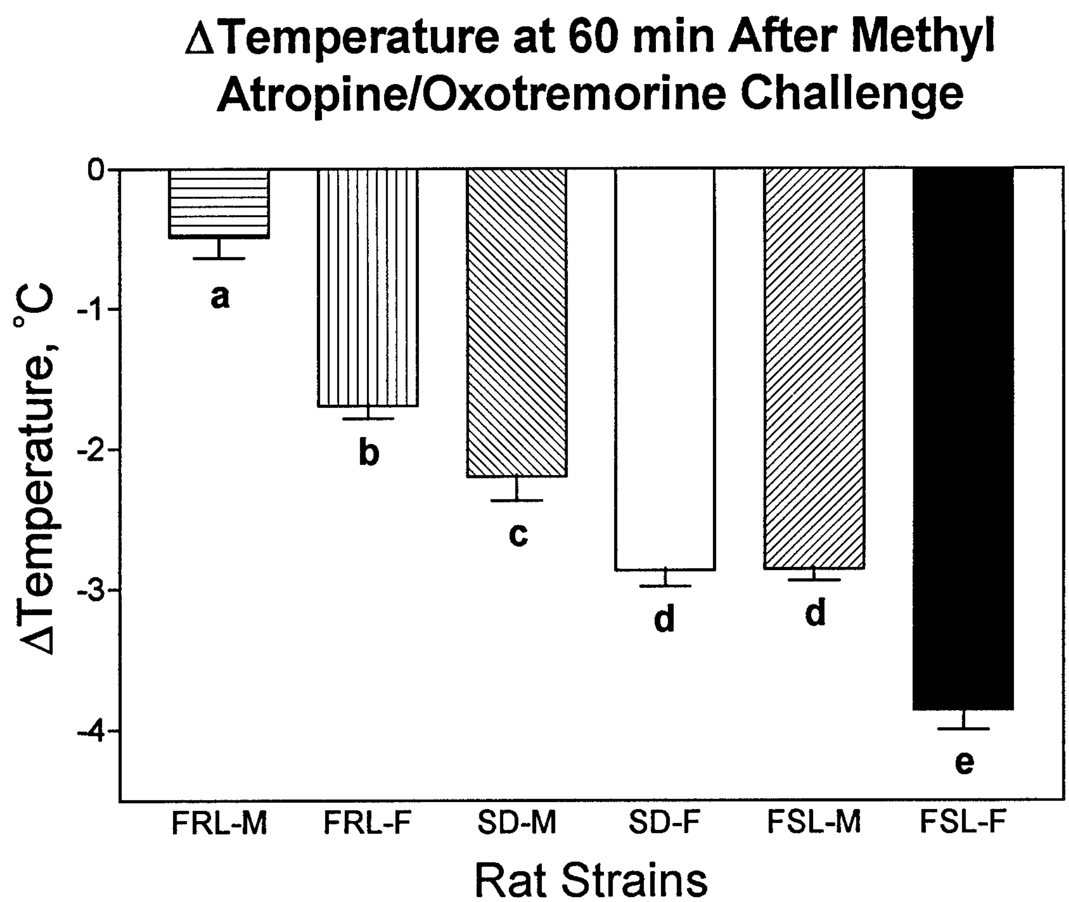
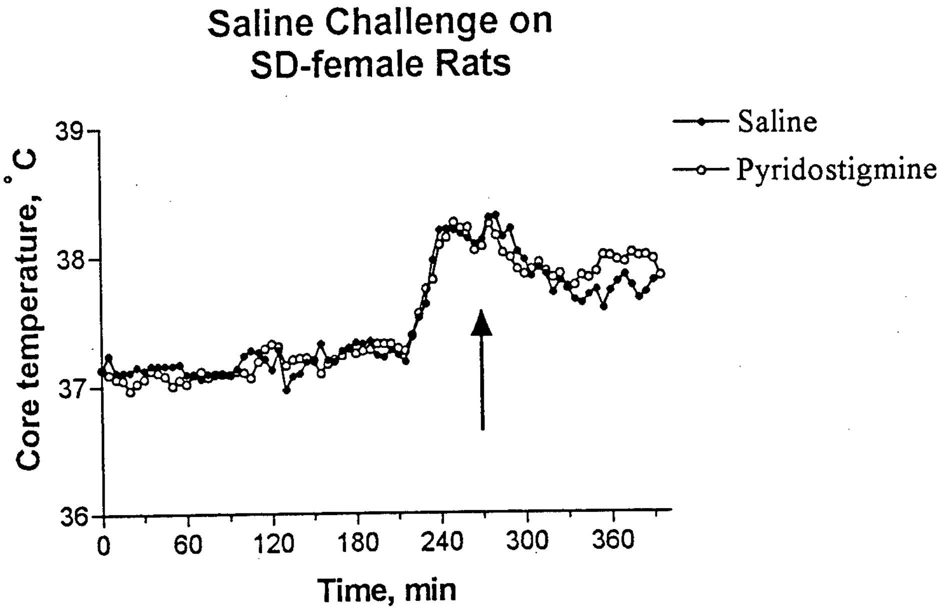
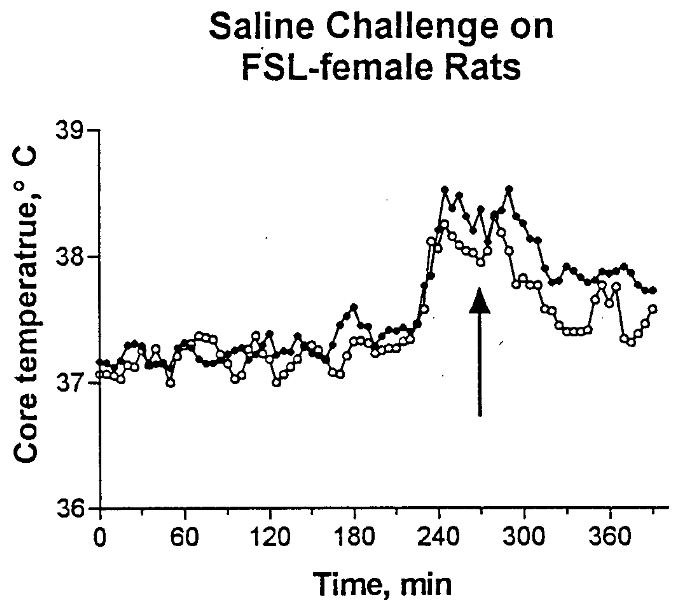

ΔTemperature at 60 min After Methyl Atropine/Oxotremorine Challenge
ΔTemperature, °C
0
-1
-2
-3
-4
a
b
c
d
d
e
FRL-M
FRL-F
SD-M
SD-F
FSL-M
FSL-F
Rat Strains

Fig. 3-1

A


Saline Challenge on
SD-female Rats
Saline
Pyridostigmine
Core temperature, °C
36
37
38
39
0
60
120
180
240
300
360
Time, min

B


Saline Challenge on
FSL-female Rats
Core temperatrue,° C
36
37
38
39
0
60
120
180
240
300
360
Time, min

C

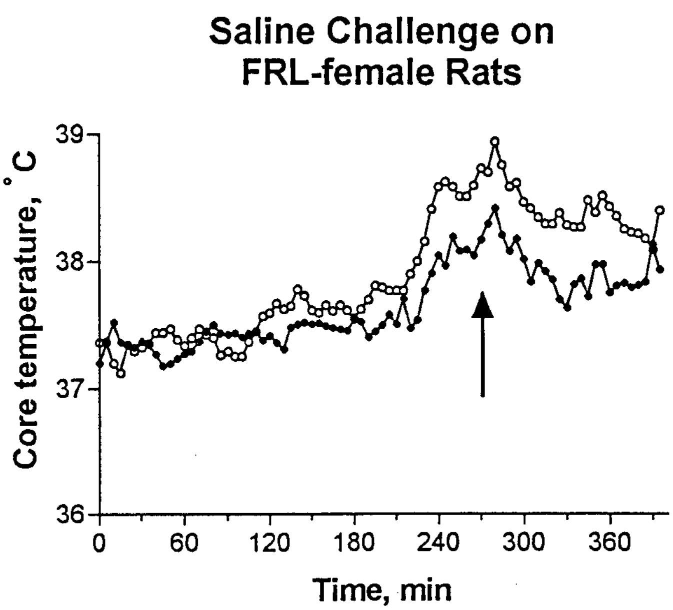
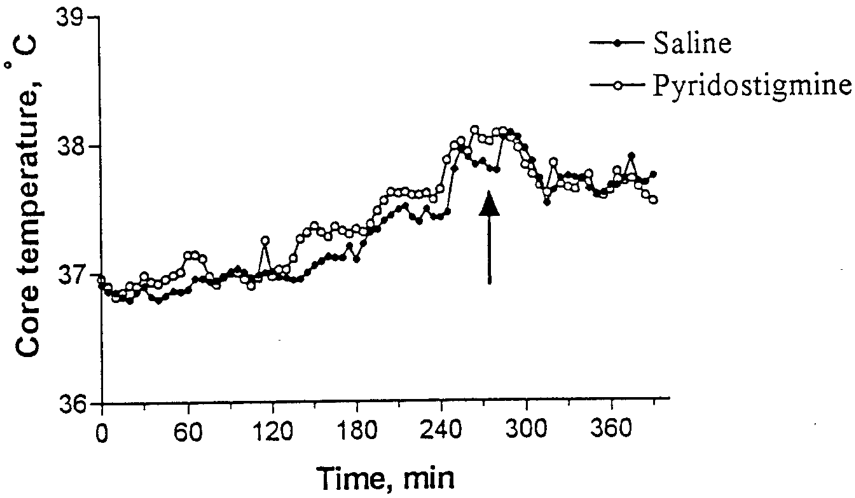

Saline Challenge on
FRL-female Rats
Core temperature, °C
36
37
38
39
0
60
120
180
240
300
360
Time, min

Fig. 3-2

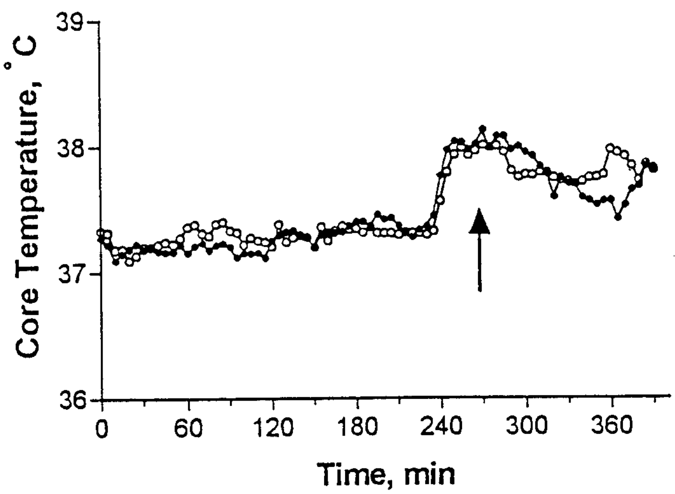
Saline Challenge on
SD-male Rats

D



Saline Challenge on
FSL-male Rats

E



Saline Challenge on
FRL-male Rats

F

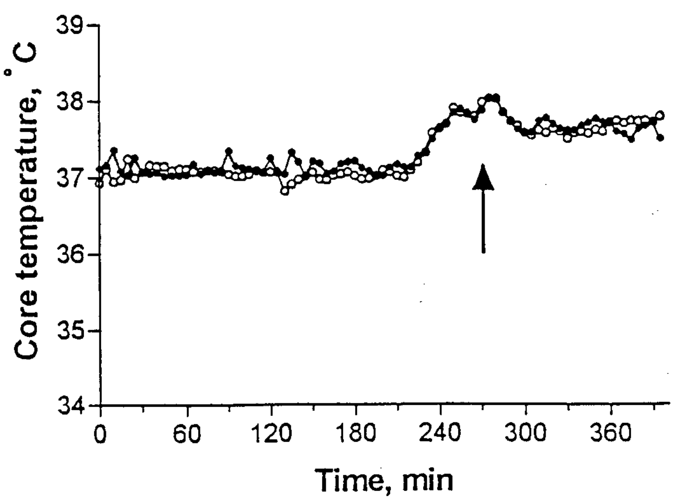


Fig. 4-1

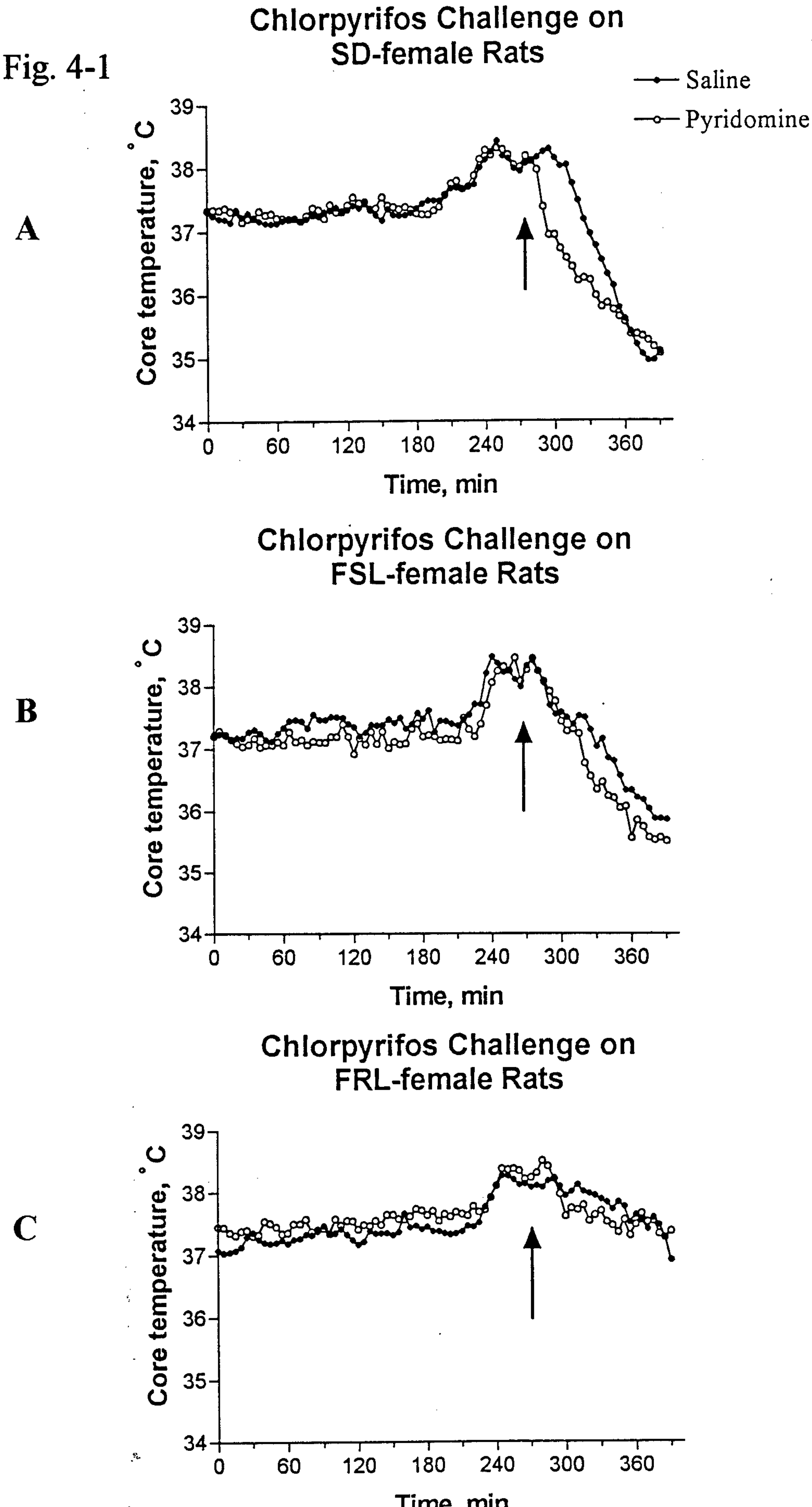
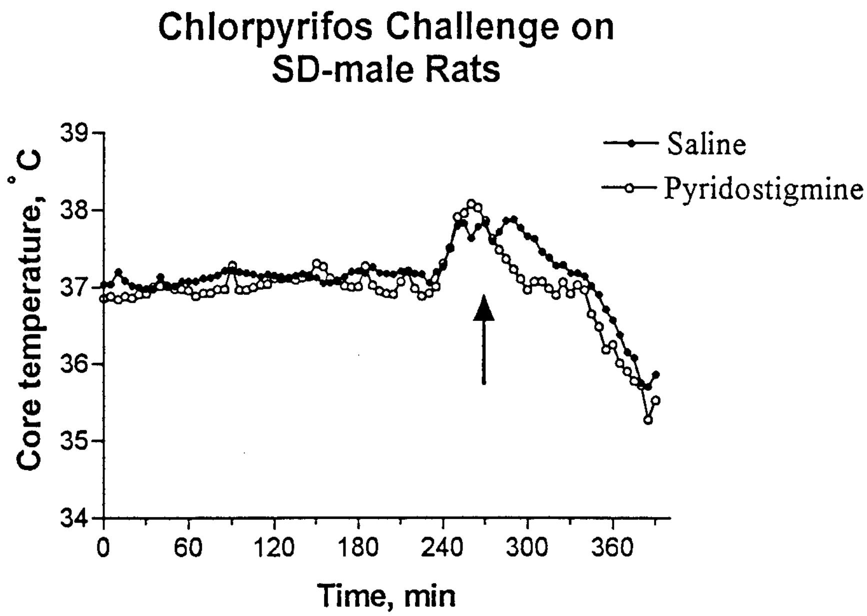
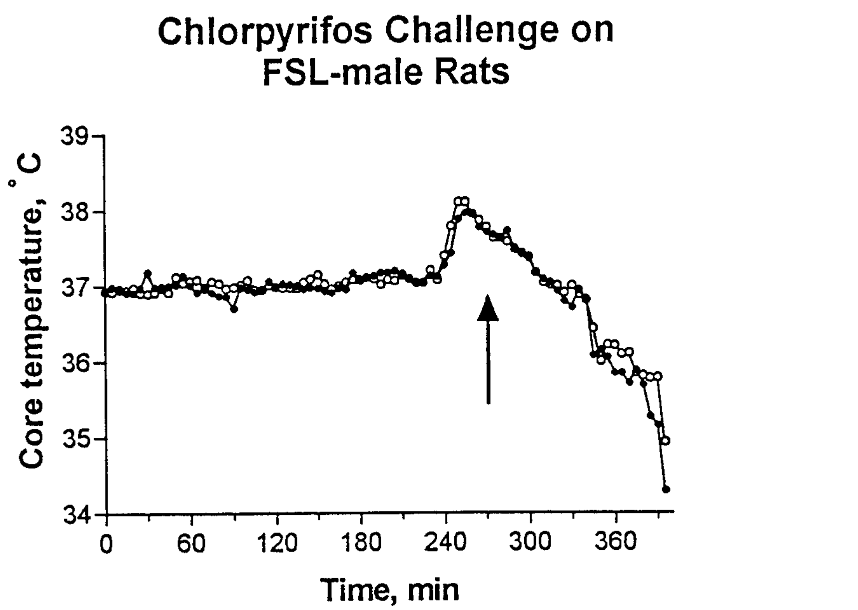

Chlorpyrifos Challenge on
SD-female Rats
Saline
Pyridomine
A
Core temperature, °C
39
38
37
36
35
34
0
60
120
180
240
300
360
Time, min
Chlorpyrifos Challenge on
FSL-female Rats
B
Core temperature, °C
39
38
37
36
35
34
0
60
120
180
240
300
360
Time, min
Chlorpyrifos Challenge on
FRL-female Rats
C
Core temperature, °C
39
38
37
36
35
34
0
60
120
180
240
300
360
Time, min

Fig. 4-2

D


Chlorpyrifos Challenge on
SD-male Rats
Saline
Pyridostigmine
Core temperature, °C
39
38
37
36
35
34
0
60
120
180
240
300
360
Time, min

E


Chlorpyrifos Challenge on
FSL-male Rats
Core temperature, °C
39
38
37
36
35
34
0
60
120
180
240
300
360
Time, min

F

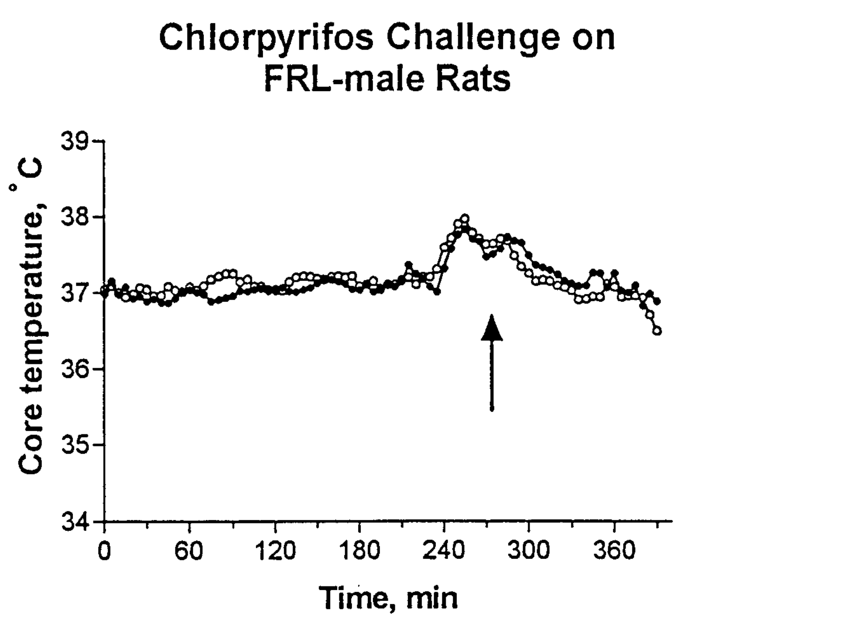
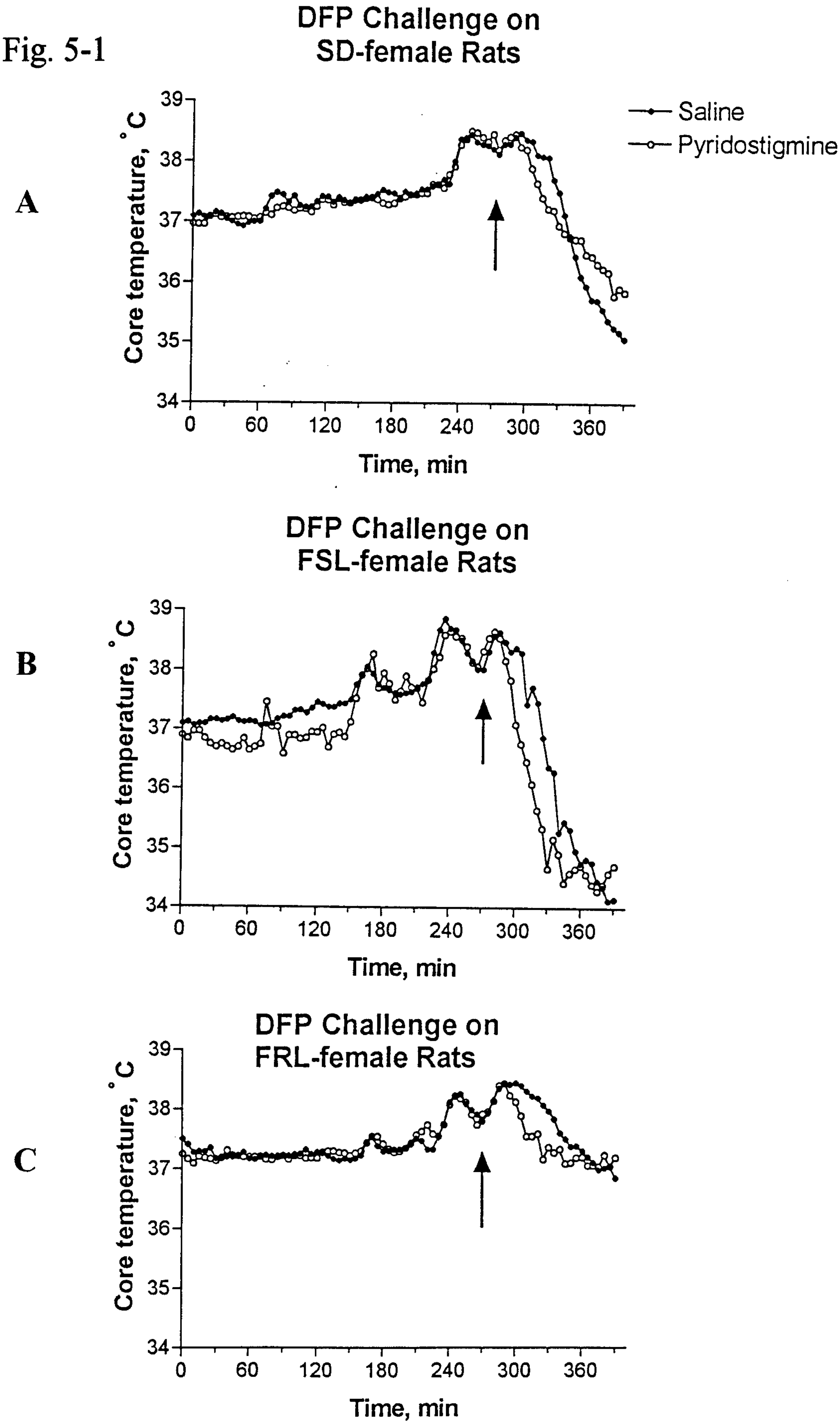

Chlorpyrifos Challenge on
FRL-male Rats
Core temperature, °C
39
38
37
36
35
34
0
60
120
180
240
300
360
Time, min

Fig. 5-1



A
DFP Challenge on
SD-female Rats
Saline
Pyridostigmine
Core temperature, °C
39
38
37
36
35
34
0
60
120
180
240
300
360
Time, min
B
DFP Challenge on
FSL-female Rats
Core temperature, °C
Time, min
C
DFP Challenge on
FRL-female Rats
Core temperature, °C
Time, min

Fig. 5-2

D

E

F

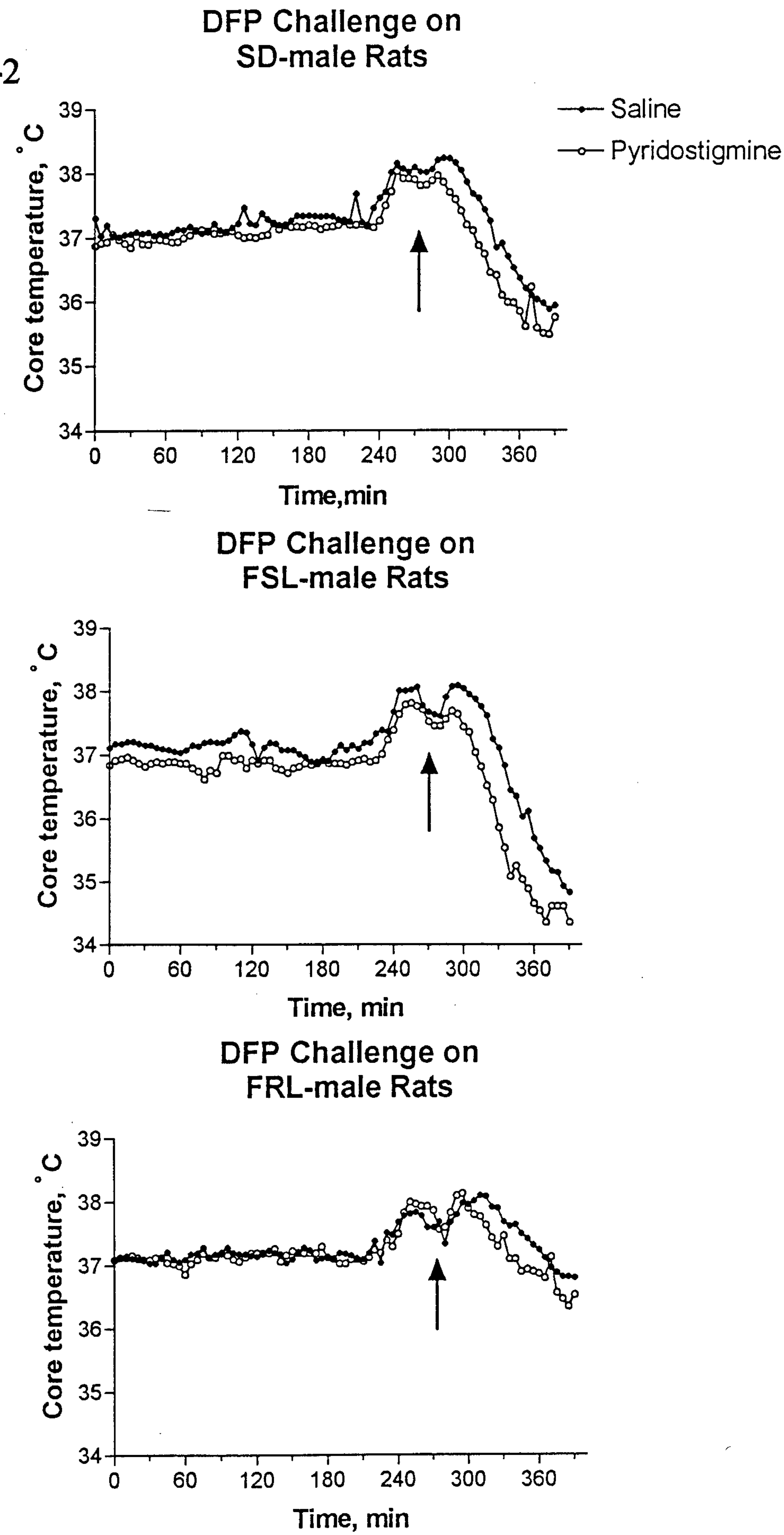
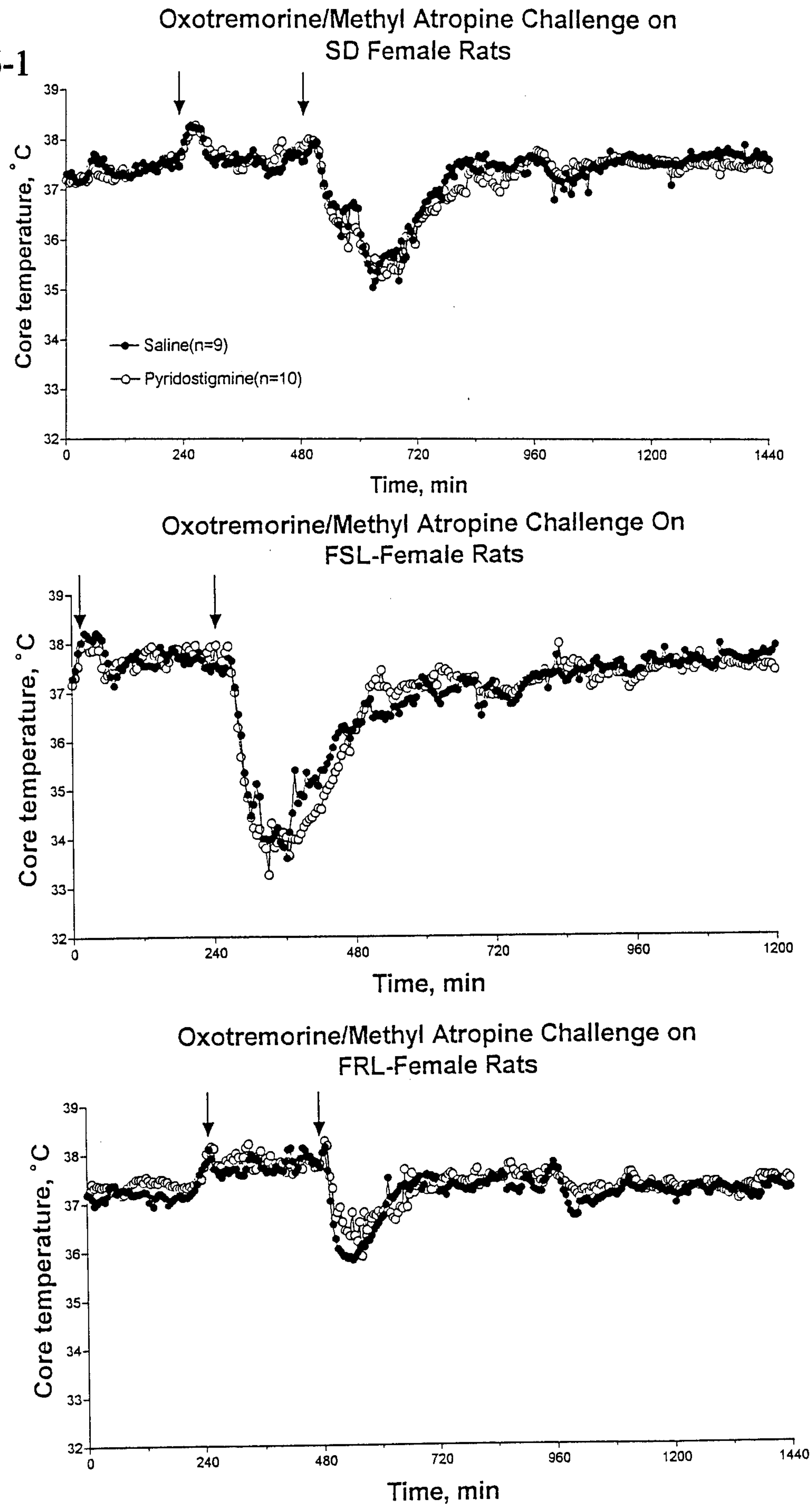

DFP Challenge on SD-male Rats
Saline
Pyridostigmine
Core temperature, °C
39
38
37
36
35
34
0 60 120 180 240 300 360
Time,min
DFP Challenge on FSL-male Rats
Core temperature, °C
Time, min
DFP Challenge on FRL-male Rats
Core temperature, °C
Time, min

Fig. 6-1

A

B

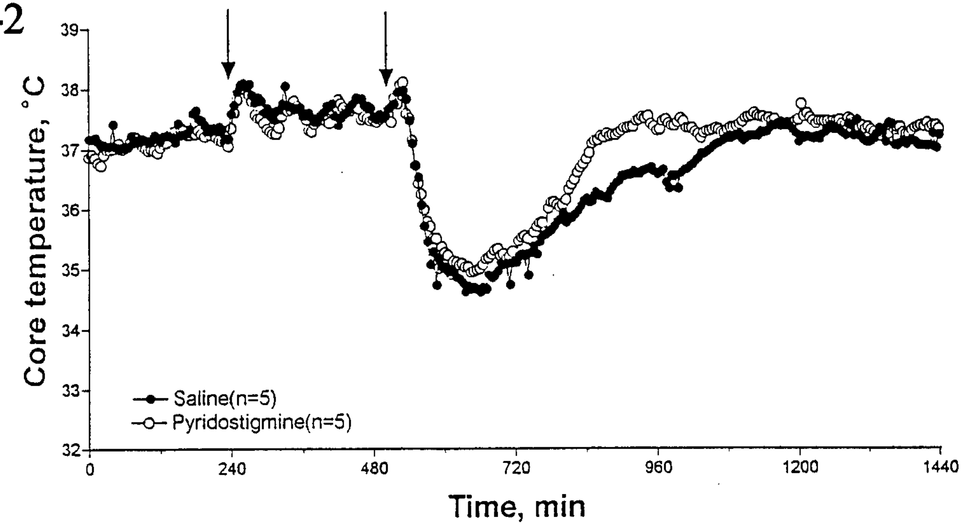
C


Oxotremorine/Methyl Atropine Challenge on
SD Female Rats
Core temperature, °C
Time, min
Saline(n=9)
Pyridostigmine(n=10)
Oxotremorine/Methyl Atropine Challenge On
FSL-Female Rats
Core temperature, °C
Time, min
Oxotremorine/Methyl Atropine Challenge on
FRL-Female Rats
Core temperature, °C
Time, min

Oxotremorine/Methyl Atropine Challenge on
SD-Male Rats

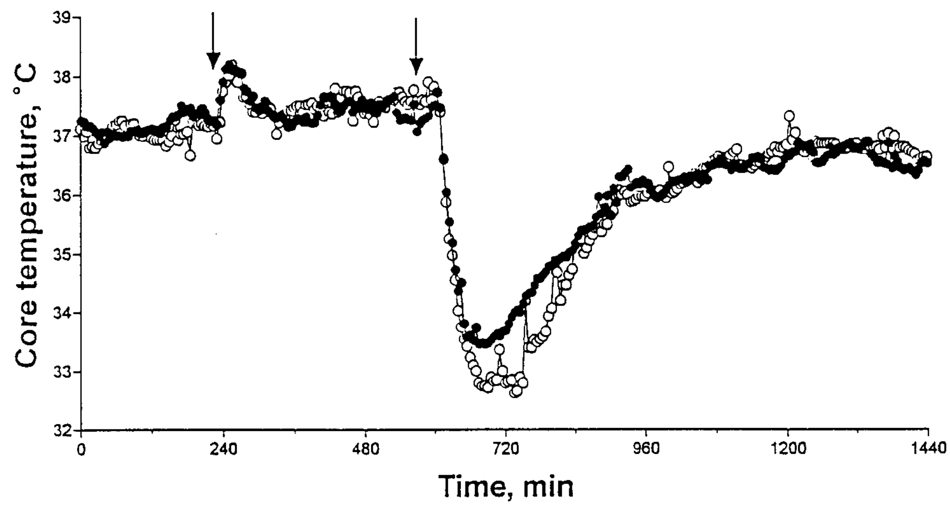
Fig. 6-2

D



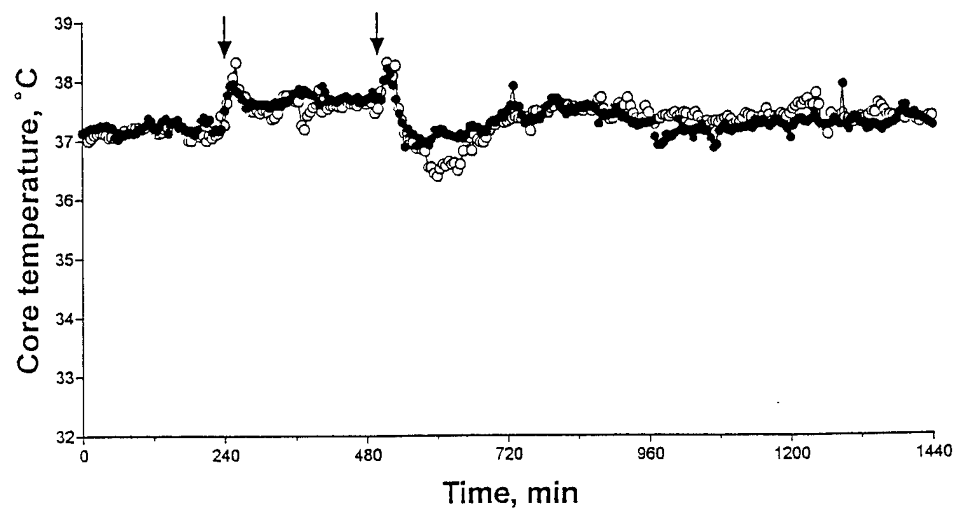
Oxotremorine/Methyl Atropine Challenge on
FSL-male Rats

E



Oxotremorine/Methyl Atropine Challenge on
FRL-Male Rats

F



CONCLUSIONS

The findings reported here are in dramatic contrast to the predominantly negative effects of pyridostigmine described in our previous annual report. The elevation of growth hormone induced by pyridostigmine was expected because of the extensive previous literature in both animals and humans. The inverted U shape function, where the levels are highest at the intermediate dose, was not anticipated, but is consistent with what has been reported for many other biological phenomena. At this time we do not have any explanation for why the level of growth hormone is not stimulated by the 36 mg/kg dose of pyridostigmine. It might be related to the general concept that if too great an inhibition of cholinesterase occurs, there is a paralysis of cholinergic transmission rather than a facilitation (Taylor, 1996). Cholinesterase assays will be performed on serums from all of the treated groups during the final year of this project and these values will be helpful in interpreting the dose-dependent effects of pyridostigmine.

The fact that growth hormone was elevated following acute pyridostigmine treatment while core body temperature and activity were not suggests that the site of the cholinergic receptors mediating the growth hormone response reside outside of the blood brain barrier. Various investigators have taken advantage of the lack of central effects of pyridostigmine to conduct challenge tests in humans (e.g. Chaudhuri et al., 1997; Ghigo et al., 1993; O'Keane et al., 1992, 1994). Unlike the centrally acting physostigmine, which induces nausea and other side effects, pyridostigmine appears to be well tolerated. We noted a very low incidence of diarrhea when the rats were sacrificed 30 min after oral pyridostigmine treatment in our first report and the current data, collected in the saline challenge rats 2.5 hour after the chronic pyridostigmine treatment, supports this.

Pretreatment with pyridostigmine chronically for 14 days significantly altered the rate of change in body temperature in the rats challenged with CPF or DFP. However, the direction of the change suggested that the rats were more sensitive to the effects of the challenge agents, not less as would have been predicted by those favoring the use of pyridostigmine as a prophylactic in Gulf War paricipants. The accelerated drop in temperature in the pyridostigmine-treated rats may be related to the blockade or saturation of peripheral cholinesterases by pyridostigmine. Since there are now fewer binding sites for DFP and CPF-oxon in the periphery, they more readily enter the central nervous system, where they will induce a decrease in body temperature. Further study of the brain cholinesterase activity in the treated rats may provide a clue to the usefulness of this argument.

Another hypothesis to account for the apparently greater sensitivity of the rats pretreated with pyridostigmine is that brain muscarinic receptors may have increased during the chronic treatment with pyridostigmine, as has been reported for the related compound neostigmine (Costa et al., 1982). Brains from each of the treatments have been stored and will be processed during the upcoming, final year of the project to determine whether there have been changes in any of the groups as a consequence of the chronic treatment regimen. These studies will also provide a comparison of the FSL and FRL rats with the SD rats. According to the data on temperature, both the FRL and FSL rats would be predicted to have muscarinic receptor differences from the SD rats.

The incidence of diarrhea was very low in all of the groups. This may have been a consequence of the mode of treatment, as the incidence was higher in the rats treated sc with DFP than in the rats treated orally with pyridostigmine or CPF. Pyridostigmine did protect against the diarrhea induced by DFP, indicating that the drug does have some prophylactic effects. Since the sites that mediate the growth hormone responses to pyridostigmine also appear to reside outside of the blood brain barrier, one might expect that the growth hormone response to CPF and DFP will also be blunted by pretreatment with pyridostigmine. Blood samples have been collected from the animals undergoing the various treatments and will analyzed for growth hormone in the upcoming year. These data will be useful in determining the effectiveness of pyridostigmine as a prophylactic against nerve agents.

In conclusion, on the basis of the present findings, pyridostigmine is not a very effective. prophylactic against centrally acting anticholinesterase agents. The decrease in temperature after CFP and DFP is more rapid and there are no differences in the peak changes at 2 hr after the injection. The 2-hr time point was selected to maximize our chances of seeing significant changes in all of the key variables we proposed to measure (temperature, growth hormone, cholinesterase activity). We have considered the possibility that 2 hr may have been too short to reveal the protective effects of pyridostigmine and will, therefore, be examining rats for up to 24 hr after their challenge treatments, when temperature is expected to recover. Our current conclusion that pyridostigmine is a relatively ineffective prophylactic against the anticholinesterase agents CPF and DFP is based on our present findings, but they are consistent with the conclusions made by other recent investigators. For example, it has been recent demonstrated that centrally acting physostigmine, administered by mnipump, was more effective than pyridostigmine in protecting against soman (Phillippens et al., 1998). We have considered the possibility of using physotigmine as a prophylactic against CPF and DFP and may conduct such an experiment in limited number of animals during the final year of this project.

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APPENDIX

The appendix consists of one manuscript (Overstreet et al., 1998) and two abstracts.

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Cholinergic/Serotonergic Interactions in Hypothermia: Implications for Rat Models of Depression

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OVERSTREET, D. H., L. C. DAWS, G. D. SCHILLER, J. ORBACH AND D. S. JANOWSKY. *Cholinergic/serotonergic interactions in hypothermia: Implications for rat models of depression.* PHARMACOL BIOCHEM BEHAV **59**(4) 777–785, 1998.—This article reviews published reports and presents new evidence that support a number of commonalties between lines of rats selectively bred for differences in cholinergic (muscarinic) and serotonergic (5-HT_{1A}) sensitivity. The Flinders Sensitive Line (FSL) rat, a genetic animal model of depression derived for cholinergic supersensitivity, is more sensitive to both cholinergic and serotonergic agonists, and exhibits exaggerated immobility in the forced swim test relative to the control, Flinders Resistant Line (FRL), rat. Similar exaggerated responses are seen in a line of rats recently selected for increased sensitivity to the 5-HT_{1A} agonist, 8-OH-DPAT (High DPAT Sensitive—HDS), relative to lines selectively bred for either low (Low DPAT Sensitive—LDS) or random (Random DPAT Sensitive—RDS) sensitivity to 8-OH-DPAT. For both the FSL and HDS rats, their exaggerated immobility in the forced swim test is reduced following chronic treatment with antidepressants. The present studies examined further the interaction between cholinergic and serotonergic systems in the above lines. Supersensitive hypothermic responses to 8-OH-DPAT were observed very early (postnatal day 18) in FSL rats, suggesting that both muscarinic and serotonergic supersensitivity are inherent characteristics of these rats. Scopolamine, a muscarinic antagonist, completely blocked the hypothermic effects of the muscarinic agonist oxotremorine in FSL and FRL rats, but had no effect on the hypothermic responses to 8-OH-DPAT, suggesting an independence of muscarinic and 5-HT_{1A} systems. On the other hand, genetic selection of genetically heterogeneous rats for differential hypothermic responses to the muscarinic agonist oxotremorine were accompanied by differential hypothermic responses to 8-OH-DPAT, suggesting an interaction between muscarinic and 5-HT_{1A} systems. Overall, these studies argue for an inherent interaction between muscarinic and 5-HT_{1A} systems, which probably occurs beyond the postsynaptic receptors, possibly at the level of G proteins. © 1998 Elsevier Science Inc.

Serotonin 8-OH-DPAT Muscarinic Oxotremorine Ontogeny FSL rat Depression Core body temperature

ALTHOUGH the underlying neurochemical components of depressive disorders are still largely unknown, it has been postulated that an interaction of, or dysbalance between, two or more neurotransmitter systems is involved [e.g., (6,26)]. Although other combinations have been put forward since Janowsky and colleagues originally proposed the cholinergic–adrenergic hypothesis of depression in 1972 (26), a hypothesis involving cholinergic–serotonergic interactions in depression has received relatively little attention to date (42,44). The present article reviews the evidence for an interaction be-

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tween serotonergic and cholinergic systems in two rat models of depression and describes new data supporting such an interaction in the regulation of temperature.

The involvement of the serotonergic system in depression and related affective disorders is now well recognized. Reports of alterations in serotonin (5-HT) receptors and/or receptor function in depressed individuals (2,6,8,31,67), downregulation of the 5-HT receptor/second messenger systems by clinically effective antidepressants [e.g. (4,18,33)], and 5-HT_{1A} receptor agonists being potentially effective antidepressants (12,34) are just a small part of the evidence that has implicated 5-HT in the pathogenesis and treatment of depression. Furthermore, serotonergic "supersensitivity" has recently been reported in depressed individuals with, for example, increased 5-HT_2 receptor function, measured as increased phosphoinositide hydrolysis in the platelets of depressed humans, occurring after 5-HT_2 agonist administration (36).

In addition, central cholinergic neurotransmitter mechanisms have long been implicated in the pathogenesis of depressive disorders (25,26,28). It is well recognized that individuals with depressive disorders are more sensitive to the behavioral (i.e., depression-inducing) and physiological (e.g., elevation of adrenocortical hormones and growth hormone, induction of REM sleep) effects of muscarinic agonists than are normal controls [e.g. (7,27,37,52,60).

With respect to cholinegic and serotonergic interactions, it has been reported that brain regions that are integral in the regulation of mood and cognition, such as the cerebral cortex and hippocampus, are rich in muscarinic receptors (mAChR) (35) and receive a dense serotonergic innervation as well (64). Pharmacological studies suggest that both systems are involved in the regulation of passive avoidance behavior (51), which might relate to depression in humans (39). Biochemical (1,19) manipulations suggest that 5-HT release may be regulated by muscarinic receptors (19), whose plasticity is dependent upon the integrity of the serotonergic system (1). Thus, not only are the cholinergic and serotonergic systems anatomically related to each other, but they also interact in such a way that a dysbalance of one system may lead to a functional deficit in the other. The etiology of affective disorders may, therefore, be attributable to a dysbalance between these neurotransmitter systems, with, for example, cholinergic overactivity predisposing to depression but subsequent alterations in serotonergic function actually inducing the depressive episodes.

Potential genetic animal models of depression have been developed by selective breeding for differential responses to muscarinic and serotonergic agonists, respectively (45–47), and the implications of these models for a cholinergic/serotonergic interaction hypothesis of depression will be the focus of the present communication. The Flinders Sensitive Line (FSL) rats represent a cholinergic model of depression (39,46). These rats were originally selectively bred to be more sensitive to anticholinesterases than the control Flinders Resistant Line (FRL) rats (41,54). However, FSL rats are also more sensitive to the behavioral and physiological effects of directly acting muscarinic agonists (39,40,46). Furthermore, FSL rats also are more sensitive to a variety of serotonergic drugs, including those that target 5-HT_{1A} and 5-HT_2 receptors (42,56,66). Preliminary experiments have reported a positive correlation between increased behavioral sensitivity and increased 5-HT receptor number in the FSL rats (56).

More recently, randomly bred, genetically heterogeneous rats were used to selectively breed for differential hypothermic responses to the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT. The line that became more sensitive to 8-OH-DPAT (the High DPAT Sensitive—HDS line) exhibited a number of similarities to the FSL rats. In addition to their supersensitive responses to 5-HT_{1A} agonists, they exhibited exaggerated immobility in the forced swim test (46,49), and this immobility could be counteracted by chronic treatment with antidepressant drugs (24,49,58). Both HDS and FSL rats also exhibit higher consumption of sweet solutions (13,47,50), and both appear to have elevated numbers of cortical 5-HT_{1A} receptor binding sites (30,47,56). Thus, there are several intriguing parallels between the HDS rats, selectively bred for increased 5-HT_{1A} sensitivity, and the FSL rats, selectively bred for increased muscarinic sensitivity.

What is particularly intriguing about these genetic animal models of depression is that although the FSL and HDS rats were selectively bred for increased hypothermic responses to oxotremorine (66) and 8-OH-DPAT (44), respectively, they exhibit similar behavioral profiles and antidepressant-like responses to clinically effective antidepressant agents (24,46,47). Table 1 summarizes these similarities among depressed individuals and the FSL and HDS rats. Note that despite several similarities, neither the HDS nor the FSL rats resemble depressed individuals in serotonergic sensitivity. The predominant approach has been to challenge depressed and control individuals with serotonergic agonists and measure specific hormones, and the most common finding is for the depressed individuals to display a blunted response (31). Hormonal responses to serotonergic challenges have not been studied in the FSL or HDS rats, but these lines are supersensitive to the hypothermic effects of 8-OH-DPAT, as mentioned above (see Table 1). Therefore, these rat models do not mimic all aspects of depressed individuals. Nevertheless, they are innately more immobile in the forced swim test and are less immobile following chronic treatment with antidepressants (Table 1).

In contrast to what appeared to occur in the FSL rats (42,66), it initially appeared that selection for differential 5-HT_{1A} sensitivity was not accompanied by a parallel increase in muscarinic sensitivity as such (45). This observation, when coupled with the results of an interbreeding study that indicated little correlation between 5-HT_{1A} and muscarinic responses in genetically heterogeneous rats (44), suggested that the serotonergic and cholinergic systems were independently regulated. In the present set of experiments we sought to obtain data that would confirm or call into question the postulated independence of, or interaction between, the serotonergic and cholinergic systems, using drug-induced hypothermia as the index variable.

TABLE 1

SIMILARITIES AMONG DEPRESSED INDIVIDUALS AND FSL AND HDS RATS

Feature/Measure	Depressed Individuals	FSL Rats	HDS Rats
Increased cholinergic sensitivity	Yes	Yes	Yes
Increased 5-HT_{1A} sensitivity	*No*	*Yes*	*Yes*
Decreased locomotor activity	*Yes*	*Yes*	*No*
Increased REM sleep	Yes	Yes	N.D.
High sweet intake craving	Yes	Yes	Yes
Immobility after stress	Yes	Yes	Yes
Antidepressant response	Yes	Yes	Yes

Areas where there is a lack of agreement are highlighted in italics.

The three approaches used were: 1) a developmental profile of 8-OH-DPAT sensitivity in the FSL and FRL rats to determine if it is similar to the developmental profile observed in FSL rats for the muscarinic agonist, oxotremorine (11); 2) a classical pharmacological blockade study of the ability of scopolamine to counteract the hypothermic effects of oxotremorine and 8-OH-DPAT; 3) a short-term selective breeding study focusing on the development of oxotremorine sensitivity, with a parallel examination of changes in 5-HT_{1A} (i.e., 8-OH-DPAT) sensitivity.

METHOD AND RESULTS

Experiment 1. Developmental Profile of 8-OH-DPAT Sensitivity in FSL and FRL Rats

Developmental profiles have shown that the FSL rats are more sensitive to the hypothermic effects of oxotremorine than are their control counterparts, the FRL rats, as early as 2 weeks postnatal, the earliest age of practical testing (10,11). Using this developmental approach, the present study aimed to compile a profile for hypothermic sensitivity to the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in FSL and FRL rats of different ages.

Animals. Male and female FSL and FRL rats aged 15, 20, 25, 31, and 60 days of age were selected from the 47th generation of FSL and FRL colonies being maintained at the Flinders University of South Australia. Body weights ranged from a mean of 29.1 ± 0.8 g (n = 36 pooled genders) at 15 days of age to 267 ± 8 g for males (n = 42) and 188 ± 4 g for females (n = 37) at 60 days of age. Data for 150- and 250-day-old rats were derived from the 43rd and 46th generations, respectively. At 250 days of age males weighed 507 ± 12 g (n = 23) and females, 309 ± 8 g (n = 21). Until the time of weaning (30 days of age) all rats were housed with respective dams and removed only during test sessions. After this time they were housed in groups of six in large metal cages with free access to food and water. The colony room was maintained at 22 ± 1°C and 50% humidity, under a 12 L:12 D cycle. The number of rats used for each test ranged between 3 and 10 of each gender. All experiments were conducted between 0800 and 1300 h. This experiment was approved by the Institutional Animal Care Committee of Flinders University.

Core body temperature recording. Core body temperature was recorded by inserting a lubricated thermocouple probe (Eirelec 5000 hand-held thermometer), 1 to 5 cm into the rectum (i.e., the distance being proportional to the age and size of the rat). Temperature was recorded to the nearest 0.1°C and was stable within 1 min after insertion of the probe. Baseline temperatures were always obtained within the 2 h preceding drug challenge. The data are typically expressed as mean ± standard error of the mean (SEM) deviations from baseline where each animal served as its own control.

Procedure. Rats were weighed and baseline core body temperatures obtained on the morning of the drug challenge tests. Animals were quasi-randomly divided into two groups and subcutaneously injected with either isotonic saline (SAL) or 8-OH-DPAT (0.1 mg/kg). Core body temperature was recorded 30 min later. The two groups were selected in such a way that there was an equal representation from each litter in the two treatment groups. The dose of 8-OH-DPAT was selected on the basis of dose–response curves for 8-OH-DPAT-induced hypothermia in adult FSL and FRL rats (57); with 0.1 mg/kg producing near maximal hypothermia and clearly delineated sensitivity differences between the two lines. To minimize the possibility of drug tolerance occurring, the treatment groups were alternated so that there was a minimum of 10 days between exposure to either 8-OH-DPAT or SAL. As an additional check for tolerance development and for further clarification of the developmental profile, a subset of rats from each litter were left drug "naive." These rats were given 8-OH-DPAT once only at selected ages (17, 18, 24, and 30 days of age). 8-OH-DPAT hydrobromide was obtained from Research Biochemicals Incorporated (Natick, MA). The dose refers to the weight of the salt and was freshly prepared on each challenge day by dissolving in isotonic saline and kept on ice to avoid degradation.

Statistical analysis. The data were subjected to multiple analysis of variance using the statistical package SPSS-X on a UNIX system mainframe computer. Where there were no significant gender differences, data for male and females were pooled. Line, age, and treatment were the factors tested. The probability level for significance was set at $p < 0.05$. Prior to any inferential statistics analyses, data were tested for homogeneity of variance using Bartlett-Box F and Cochran's C-tests. Both confirmed homogeneity of variance.

Results. Within each line there were no significant gender differences in change in core body temperature after 8-OH-DPAT; therefore, the data for males and females were pooled. The results depicted in Fig. 1 highlight the pronounced differences in sensitivity between the FSL and FRL rats with respect to 8-OH-DPAT–induced hypothermia. The FSL rats displayed a significantly greater 8-OH-DPAT–induced hypothermic effect than the FRL rats at all ages tested with the exception of hypothermia at 15 days of age, where the lines were not different. This yielded a significant main effect of line, $F(1, 276) = 184.64$, $p < 0.001$. The magnitude of this difference varied with age, being maximal at 18 and 31 days of age, and yielded a significant main effect of age, $F(9, 276) = 43.88$, $p < 0.001$. Furthermore, an interaction effect between line and age was established (Fig. 1). FSL, relative to FRL rats, became more sensitive to the hypothermic effect of 8-OH-DPAT with age [line × age, $F(9, 276) = 5.87$, $p < 0.001$].

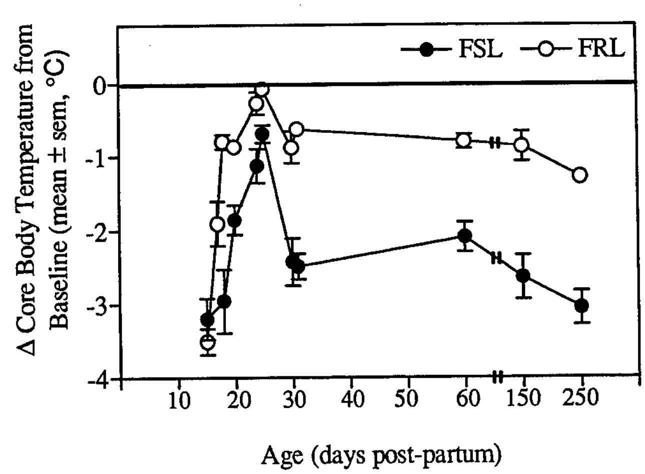

FIG. 1. Age-dependent changes in mean core body temperature after 0.1 mg/kg (SC) 8-OH DPAT. Data for male and female rats were pooled because no significant gender differences were established with respect to drug-induced change in core body temperature. There were 6 to 20 rats per group. Each animal served as its own control and change in temperature is with respect to normal baseline core body temperature.

8-OH-DPAT "naive" rats, depicted at 17, 18, 24, and 30 days of age in Fig. 1, did not deviate from the general developmental pattern observed in rats that received 8-OH-DPAT on three separate occasions at 10-day intervals.

Saline-injected controls exhibited minor fluctuations about a mean of 0°C change in core body temperature over all ages tested (data not shown), with the extreme ranges being from −0.4 to +0.2°C.

Experiment 2: Pharmacological Blockade of 8-OH-DPAT–Induced Hypothermia

This experiment explored the possibility that 5-HT_{1A} sensitivity, as shown by hypothermia, is caused by muscarinic sensitivity in the Flinders Line rats due to serotonergic neurons synapsing on cholinergic neurons, which transmit to the heat loss pathways. If this model of cholinergic neurons being the final common path to hypothermia is correct, then scopolamine, a centrally acting muscarinic antagonist, should block or partially counteract the hypothermic effect of 8-OH-DPAT, the 5-HT_{1A} receptor agonist, as well as that of oxotremorine, the muscarinic agonist. Experiments were conducted in adult FSL and FRL rats to investigate this hypothesis.

Animals. Male and female FSL and FRL rats were selected from the 48th generation of the breeding colonies maintained at Flinders University. The rats were between 75–80 days old at the beginning of the study and weighed approximately 350 g (for males) or 205 g (for females). The rats were housed and maintained as described above. This experiment was approved by the Institutional Animal Care Committee of Flinders University.

Procedure. The rats were randomly divided into eight treatment groups so that there was an even representation of litter mates in each group (7–16 rats per group). The groups received either isotonic saline (four groups) or scopolamine (0.2 mg/kg, four groups) pretreatments followed by saline, oxotremorine (0.19 mg/kg), or 8-OH-DPAT (0.1 or 0.5 mg/kg) 15 min later. Scopolamine hydrochloride and oxotremorine sesquifumarate were obtained from Sigma (St. Louis, MO), and 8-OH-DPAT was obtained from Research Biochemicals Incorporated (Natick, MA); doses refer to the salts for the drugs. Core body temperatures were recorded at baseline, when the animals were weighed, and at 30 min after the final injection. All injections were given SC in 1 ml/kg.

Statistical analyses. Results are expressed as mean ± SEM changes in °C from the corresponding baseline measures. The data were subjected to two-way ANOVAs, with line and treatment as the main factors. Post hoc analyses were performed using Scheffe's multiple contrast tests. Where appropriate, *t*-tests were performed to test the significance of selected pairs of data.

Results. The effects of scopolamine pretreatment on oxotremorine- and 8-OH-DPAT–induced hypothermia in the FSL and FRL rats are illustrated in Fig. 2. Two-way ANOVA indicated highly significant treatment ($F = 80.7$, $p < 0.001$) and line ($F = 142.8$, $p < 0.001$) effects. The saline vehicle produced a small hyperthermic response, while scopolamine slightly reduced temperature in the FSL rats and increased it in the FRL rats (Fig. 2A). Scopolamine significantly counteracted the decrease in body temperature induced by oxotremorine in both lines, as expected (Fig. 2A). In contrast, the hypothermia induced by either dose of 8-OH-DPAT was only slightly affected by scopolamine pretreatment (Fig. 2B). Thus, these findings indicate that scopolamine selectively blocks hypothermia induced by the muscarinic receptor agonist, oxotremorine.

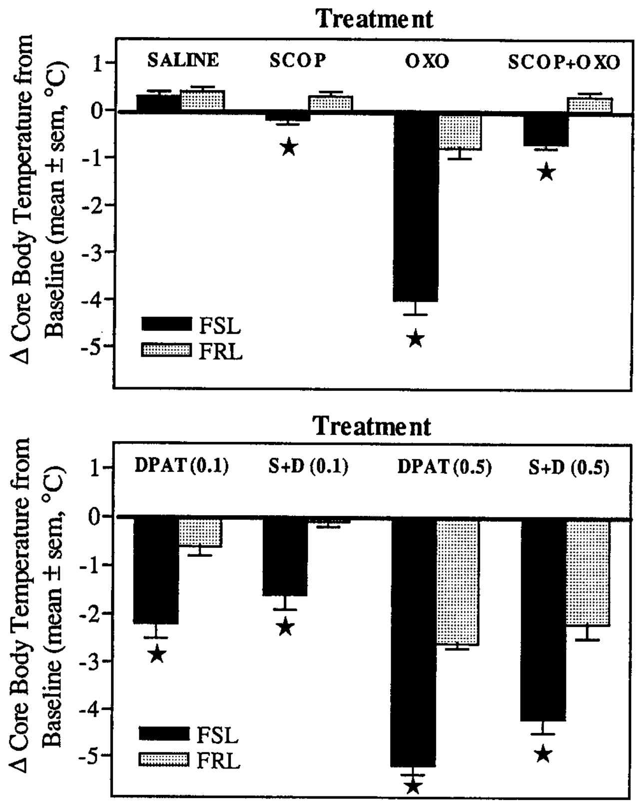

FIG. 2. Scopolamine blockade of hypothermia induced by oxotremorine but not 8-OH-DPAT. Scopolamine (0.2 mg/kg) or saline vehicle was given SC 15 min prior to the administration of saline vehicle, 8-OH-DPAT (0.1 or 0.5 mg/kg), or oxotremorine (0.19 mg/kg). Data represent the mean ± SEM changes in °C from baseline for 7–16 rats.

Experiment 3: Genetic Selection for Differential Hypothermic Responses to Oxotremorine

When the serotonergic sensitivity differences were first discovered in the FSL and FRL rats (66), 13 generations of selection for muscarinic sensitivity had occurred. Consequently, the apparent association between muscarinic and 5-HT_{1A} sensitivity may have occurred by chance and not by a genetic correlation. The fact that muscarinic and 5-HT_{1A} sensitivities are not significantly correlated in the previously mentioned intercross experiments between the FSL and FRL rats (43) would appear to support the lack of close genetic association between the cholinergic and serotonergic sensitivity. However, we have found dramatic differences in muscarinic sensitivity to oxotremorine in later generations of the randomly bred genetically heterogeneous rats that were selectively bred for differential hypothermic responses to 8-OH-DPAT, despite only small differences in the earlier generations (43,45). These studies thus provide mixed support for the hypothesis of a genetic association between muscarinic and 5-HT_{1A} sensitivities.

The present experiment sought to clarify this relationship by using randomly bred genetically heterogeneous rats to conduct a short-term selective breeding study in which serotonergic and cholinergic sensitivity were simultaneously evaluated in rats bred for differences in oxotremorine sensitivity.

Animals. The animals were selected from a genetically heterogeneous (N/Nih) breeding colony that was established in the Center for Alcohol Studies at the University of North Carolina (45). Since obtaining breeding stock from NIH, these rats have been maintained by breeding 10 pairs per generation, with no matings occurring between close relatives. Litter size averages 10–12 rats. The rats were housed in standard housing conditions under a reversed 12 L:12 D cycle, with lights off between 1000 and 2200 h. This experiment was approved by the Institutional Animal Care Committee of the University of North Carolina.

Procedure. The study began with an oxotremorine challenge at weaning (28–32 days of age). The rats were marked, weighed, and baseline temperatures were recorded with a rectal thermocouple probe attached to a Sensortek digital thermometer. They were then injected SC with a drug mixture containing oxotremorine (0.2 mg/kg) and atropine methyl nitrate (2 mg/kg). Core temperatures were recorded 30 min later and rats were selected for breeding according to their hypothermic responses. The male and female rat from each litter that exhibited the greatest decrease in temperature were used to establish the High Oxotremorine Sensitivity (HOS) group; those that exhibited the smallest decrease in temperature were used to establish the Low Oxotremorine Sensitivity (LOS) group.

Animals were paired for mating so that there were no close relatives. In the first generation progeny, the same procedures as described above were carried out at weaning: handling, weighing, recording of baseline temperature, injection of oxotremorine/methyl atropine mixture, recording of temperature at 30 min. Again, the most affected male and female were used to continue the HOS line and the least affected male and female were used to continue the LOS line.

The same procedure was followed once again for the second generation progeny, with one addition. Approximately 5 days after the oxotremorine challenge, the rats were given a single 0.5 mg/kg SC injection of 8-OH-DPAT, and core temperature was recorded 45 min later, as is typically done in the 8-OH-DPAT–selected rats (45,47).

To provide a reference group, data from the 7th generation of the HDS and LDS rats, selected for their differential hypothermic responses to 8-OH-DPAT, were included. The rats were first given 8-OH-DPAT (0.5 mg/kg, SC) at weaning and their temperatures recorded 45 min later. Then, approximately 5 days later, they were challenged with a mixture of oxotremorine and methyl atropine, as described above, and temperatures recorded 30 min later. Finally, in addition to the LOS and HOS groups, selectively bred for differences in oxotremorine-induced hypothermia, and the HDS and LDS groups, selectively bred for differences in 8-OH-DPAT–induced hypothermia, a group of randomly bred genetically heterogeneous (RDS) rats were included.

Oxotremorine sesquifumarate and atropine methyl nitrate were obtained from Sigma (St. Louis, MO) and 8-OH-DPAT was obtained from Research Biochemicals Incorporated (Natick, MA). Doses refer to the salts of the respective drugs.

Statistical analyses. The data were analyzed by one-way ANOVAs, with follow-up Newman–Keuls tests.

Results. Hypothermia induced by oxotremorine or 8-OH-DPAT was studied in similarly maintained animals from the 7th generation of selection of the 8-OH-DPAT–selected lines (HDS and LDS) and the 2nd generation of selection of the oxotremorine-selected lines (HOS and LOS). There were highly significant ($F = 57.18$, $p < 0.0001$) differences among the groups. In Fig. 3, it can be seen that there appeared to be rapid selection for the differential hypothermic response to oxotremorine, as the HOS rats exhibited a significantly greater hypothermic response than the LOS rats, with the randomly bred (RDS) rats intermediate. This figure also shows that there are significant differences in the hypothermic response to oxotremorine in the HDS and LDS rats, selectively bred for differential hypothermic responses to 8-OH-DPAT. The HDS rats were more sensitive to oxotremorine.

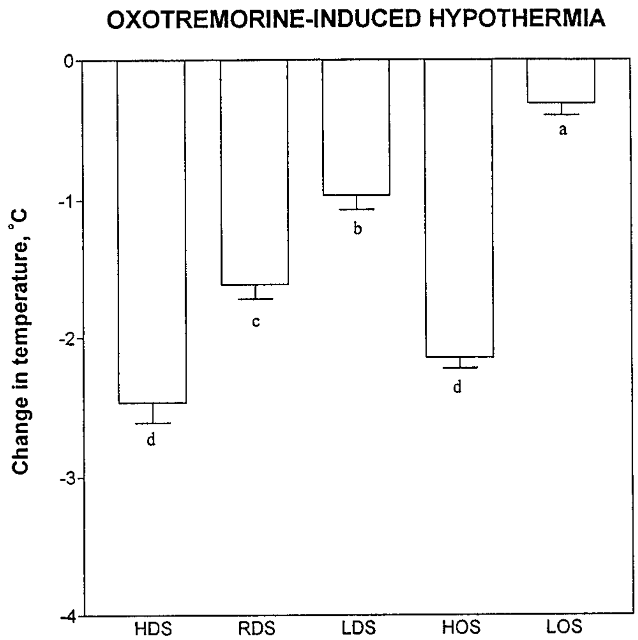

FIG. 3. Hypothermia induced by oxotremorine in rats selectively bred for differential hypothermic responses to oxotremorine or 8-OH-DPAT. A mixture of oxotremorine (0.2 mg/kg) and methyl atropine (2 mg/kg) was administered 30 min prior to the recording of core temperature by a rectal thermistor probe. The data represent the mean ± SEM changes in °C from baseline for 17–36 male and female rats. Groups with different letters are significantly different, $p < 0.01$, Newman–Keuls test.

The converse data, illustrated in Fig. 4, present a rather similar picture. There are large and significant differences in hypothermic responses to 8-OH-DPAT in the lines selectively bred for differential responses to this agent (HDS and LDS), but there are also significant differences between the HOS and LOS lines, selectively bred for differences in oxotremorine-induced hypothermia ($F = 100.61$, $p < 0.001$). The HOS line is more sensitive to the hypothermic effects induced by 8-OH-DPAT. As for oxotremorine, the randomly bred RDS rats are intermediate between the HDS and LDS rats.

GENERAL DISCUSSION

The present findings, utilizing three diverse approaches, argue for an interaction between the cholinergic and serotonergic systems in these rat models of depression. However, the hypothesis that simple changes in 5-HT_{1A} or muscarinic receptors can account for the present findings cannot be supported, and alternative mechanisms must be considered.

Experiment 1 demonstrated that both FSL and FRL rats exhibited a hypothermic response to 8-OH-DPAT at the earliest age tested, suggesting that the 5-HT_{1A} system is already

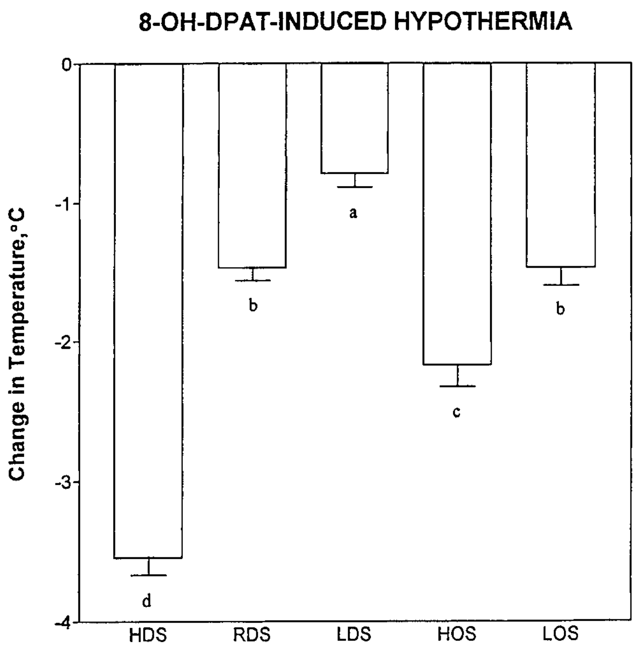

FIG. 4. Hypothermia induced by 8-OH-DPAT in rats selectively bred for differential hypothermic responses to oxotremorine or 8-OH-DPAT. 8-OH-DPAT (0.5 mg/kg) was administered 45 min prior to the recording of core temperature by a rectal thermistor probe. The data represent the mean ± SEM changes in °C from baseline for 20–31 male and female rats. Groups with different letters are significantly different, $p < 0.01$, Newman–Keuls test.

functional at 15 days of age. The ability of 8-OH-DPAT to exert an effect early in life is in good agreement with the literature (14,29,53,62,63). The present results are further supported by the observation that saline did not result in a significant change in core body temperature from baseline. The ability of saline-treated pups to maintain a constant body temperature when separated from their respective dams for extended periods of time indicates that the capacity to thermoregulate efficiently is present at 15 days of age and thus is not a confounding variable.

Because the FRL rats were considered control rats for the developmental (i.e., ontogenetic) study, it is appropriate to first compare the data derived from FRL rats with those in the literature for randomly bred Sprague–Dawley rats. There is a paucity of literature regarding 8-OH-DPAT–induced hypothermia in juvenile rats. However, the decrease in core body temperature (approx. −1.0°C) exhibited by adult FRL rats is comparable to reports where data have been obtained under similar conditions [e.g., (17,21,22)]. The greater hypothermia (−2.0 to 3.5°C) observed in rats aged 15–17 days is perhaps not surprising, because sensitivity to various serotonergic and cholinergic drugs, quantified using a variety of behavioral measures, have been reported to alter with age (53,61). For example, the 5-HT antagonist, metergoline, inhibited suckling in 3–4- and 7–8-day-old rat pups but had little influence on suckling behavior in older 21–24-day-old pups (53). Receptor number and/or affinity, coupling to second messengers, and integration of neurotransmitter systems often undergo dynamic changes during the first 2–3 postnatal weeks. These changes may not solely serve as precursors for the adult neurotransmitter systems but may also be related to the mediation of behaviors essential to the young pup (53). In summary, the FRL rats responded to a pharmacological manipulation of the serotonergic system that closely resembled that described in the literature. Any gross deviation from this ontogenetic profile exhibited by the FSL rats is, therefore, very likely to be a trait of these selectively bred rats.

Comparison of the developmental profiles for FSL and FRL rats revealed marked differences in their sensitivity to 8-OH-DPAT. FSL rats older than 15 days were supersensitive to the hypothermic effect of 8-OH-DPAT. Thus, 5-HT_{1A} supersensitivity, like muscarinic supersensitivity (10), appears to be an inherent characteristic of the FSL rats. Figure 1 illustrates the virtually parallel development of sensitivity to 8-OH-DPAT–induced hypothermia in FSL compared to FRL rats. The FSL rats, aside from exhibiting greater hypothermia, did not deviate from the ontogenetic pattern of the FRL rats. Both lines were least sensitive to the hypothermic effect of 8-OH-DPAT at 25 days of age. Sensitivity to 8-OH-DPAT then increased quite rapidly, and levels of adult responsivity were reached at 30 days of age. This pattern was not attributable to the emergence of tolerance to 8-OH-DPAT, because age-matched 8-OH-DPAT naive rats exhibited a hypothermia that fell within the bounds of the developmental profile derived from the main experimental group that received 8-OH-DPAT on three separate occasions. One functional implication of such change in sensitivity during development, as described earlier, may be the need for the suppression of neonate behaviors as adult behaviors emerge (55). The underlying neurochemical basis(es) for this period of relative insensitivity cannot be determined from these data alone, and so we can only speculate. However, it is noteworthy that the ontogenetic profile for sensitivity to the muscarinic agonist oxotremorine was similar in shape but the period of relative insensitivity occurred earlier (18 days of age) than for 8-OH-DPAT in FSL rats [(11); see Fig. 5].

Transient periods of altered sensitivity to drugs do not appear to be unique to a single neurotransmitter system and are probably attributable to the dynamic changes that take place during synaptogenesis in the early postnatal weeks. For example, the efficacy of receptor–second messenger coupling (5) and/or expression of genes involved in the manufacture of the various enzymes and receptors that combine to form the functional adult neural network undergo marked changes during the early period in development (19,22,23). The 5-HT_{1A} receptor gene is one such example. A high rate of expression is observed during a limited period in fetal life and again at 18 days of age, after which time the level of gene expression falls quite markedly (20). It is tempting to postulate that after 25 days of age, the FSL rats undergo a further period of 5-HT receptor gene overexpression, which may, at least in part, explain their "adult" supersensitivity to 8 OH-DPAT. Indeed, this is an appealing hypothesis, because preliminary receptor binding studies have indicated an approximately 20% increase in 5-HTlA receptor number in adult FSL compared to FRL rats (56) and in the HDS compared to LDS rats (30,47).

Although the neurochemical and/or metabolic changes that occur early in development are complex, it is clear that the FSL and FRL rats are different in their response to both 8-OH-DPAT and oxotremorine [(10,11); Fig. 5]. The most striking difference is the enhanced sensitivity of FSL rats to the hypothermic effect of these agonists. A more subtle difference, highlighted in Fig. 5, is the ontogenetic time frame for the periods of relative insensitivity to the hypothermic effect of 8-OH-DPAT and oxotremorine. As discussed earlier, both FSL and FRL rats are least sensitive to 8-OH-DPAT at 25

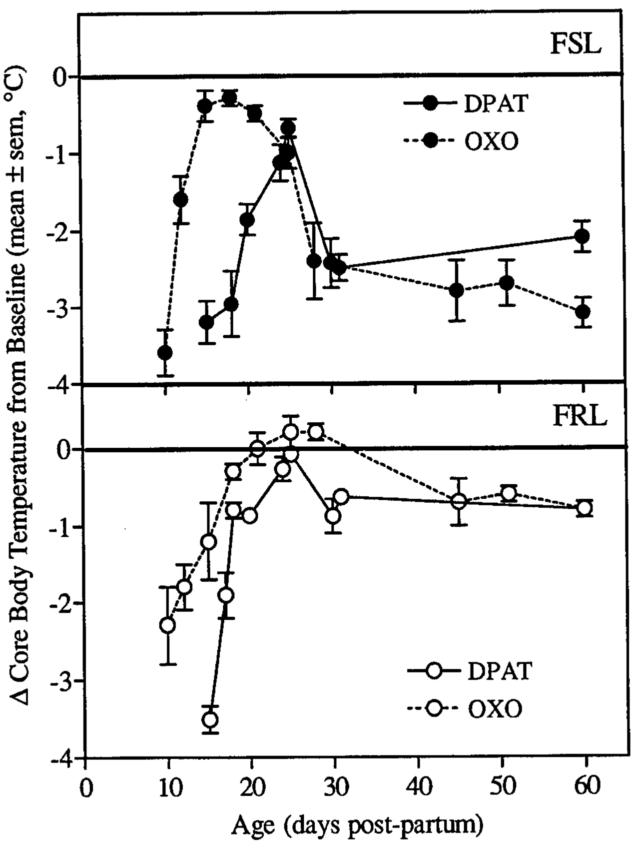

FIG. 5. A comparison of age-dependent changes in mean core body temperature after 0.1 mg/kg (SC) 8-OH-DPAT or 0.25 μmol/kg (SC) oxotremorine. Data for 8-OH-DPAT is duplicated from Fig. 1 and data for oxotremorine is modified from Daws and Overstreet (submitted). Data for male and female rats (within a line) were pooled because no significant gender differences were established with respect to drug-induced change in core body temperature. There were 5 to 20 rats per group. Each animal served as its own control and change in temperature is with respect to normal baseline core body temperature. Associated standard error of the means were not greater than 0.4°C and have been omitted for the sake of clarity. Solid symbols = FSL rats, open symbols = FRL rats; circles = 8-OH-DPAT, squares = oxotremorine.

days of age. The FRL rats are also least sensitive to oxotremorine-induced hypothermia at 25 days of age. This contrasts with the FSL rats, where the greatest insensitivity to oxotremorine-induced hypothermia occurs at 18 days of age. Thus, there are inherent differences not only in the sensitivity of these rat lines to muscarinic and serotonergic agonists, but also in the nature of their developmental profiles. Recent cross-breeding studies using the FSL and FRL rats suggest that muscarinic sensitivity is under the influence of additive and dominance genetic factors, whereas serotonergic sensitivity appears to be influenced by solely additive genetic factors (43,45). Together with the findings of the developmental studies, it appears that both cholinergic and serotonergic systems are instrumental in determining the altered phenotype of the FSL rats.

There is a growing body of literature regarding the genetics of affective disorder in humans (15), and recent studies have shown serotonergic dysfunction in prepubertal major depression patients (55) as well as cholinergic supersensitivity in children at risk for depression (59). The early emergence of serotonergic supersensitivity in the FSL rats provides yet another characteristic to add to the growing suite of parallels between the FSL rats and human depressives (39). Thus, the FSL animal model of conjoint cholinergic/serotonergic supersensitivity may well be heuristic in understanding the neurochemical causes of depressive illness, particularly with respect to a cholinergic–serotonergic balance hypothesis.

Unfortunately, there have been no studies to date that have examined cholinergic or serotonergic drugs on temperature regulation in humans despite its relative noninvasiveness. Instead, human studies have more often focused on changes in neuroendocrine or sleep measures after challenge with serotonergic or cholinergic agents (9,28,31). Also, none of these studies have used both serotonergic and cholinergic probes in the same group of subjects, so it is impossible to assess the value of the cholinergic serotonergic balance hypothesis at this time. The studies on the rat models presented here argue strongly for the necessity of such parallel studies in humans.

Experiment 2 demonstrated that scopolamine blocked the hypothermic responses induced by the muscarinic agonist oxotremorine but not the 5-HT_{1A} agonist 8-OH-DPAT (Fig. 2). Therefore, the serotonergic system probably is not linked in series with the cholinergic system in inducing hypothermia. There is other evidence from selectively bred rat lines that supersensitive hypothermic responses may not be dependent exclusively on changes in muscarinic or 5-HT_{1A} receptors. The supersensitive muscarinic response can be seen very early (Fig. 5), but the muscarinic receptor elevations in the hypothalamus do not appear until 60 days of age (10). Despite the large differences in hypothermic responses to 8-OH-DPAT in the HDS and LDS lines, there are no differences in hypothalamic 5-HT_{1A} receptors in these lines (30). Thus, the close developmental profiles of oxotremorine and 8-OH DPAT sensitivity and the parallel changes occurring during selective breeding must be accounted for by mechanisms other than simple changes in receptors.

However, the mechanism must be closely related to both cholinergic and serotonergic systems because, as Experiment 3 demonstrates, there were parallel changes in both oxotremorine- and 8-OH-DPAT–induced hypothermic responses when animals were selectively bred for differences in oxotremorine sensitivity (Fig. 3). This study provided, therefore, confirmatory evidence for the association of 5-HT_{1A} and muscarinic supersensitivity in the FSL rats (42,56,57,64). This experiment also demonstrated that the lines selectively bred for differential hypothermic responses to 8-OH-DPAT, the HDS and LDS rats, are now also differentially sensitive to the muscarinic agonist, oxotremorine (Fig. 4). These parallel changes in muscarinic and 5-HT_{1A} sensitivity during selective breeding for either muscarinic or 5-HT_{1A} sensitivity argue strongly for a common underlying mechanism.

One potential mechanism that could account for the above observation is an alteration in G proteins or in some other aspect of the second-messenger systems. According to various biochemical and molecular studies, both muscarinic M2 receptors and 5-HT_{1A} receptors interact with a Gi protein which contributes to the inhibition of cyclic AMP (38). In contrast, the muscarinic M1 receptor and the $5\text{-HT}_2\text{A}$ receptor are positively linked to the phosphatidyl inositol second-messenger system (38). It is not clear at present whether the hypothermic effects of oxotremorine or OH-DPAT are mediated through a Gi protein. However, if they were and these proteins changed as a consequence of selective breeding, then the parallel changes in 5-HT_{1A} and muscarinic sensitivity could be explained.

Furthermore, there is also considerable interest in the possibility that G proteins may be involved in the etiology and phenomenology of depression in humans (3,48), and that alterations in G protein function may accompany chronic treatment with antidepressant drugs (3,32,33). Thus, it is possible that both FSL and HDS rats exhibit exaggerated immobility in the forced swim test and other behavioral analogs of depression because selective breeding for the increased hypothermic responses to their respective drugs has resulted in a similar change in G protein function. An investigation of this hypothesis in the FSL and HDS models of depression may, in turn, help clarify the mechanisms underlying human depression, in particular, the growing body of evidence implicating altered G protein function in affective disorders (3,32,33,48).

This article would not do justice to the impressive literature on depressive disorders if it did not conclude with this cautionary note. Although almost all of the available evidence accumulated to date is consistent with cholinergic supersensitivity in depressive disorders (28), there is a wealth of information suggesting an association of serotonergic subsensitivity with affective disorders, and not serotonergic supersensitivity as indicated above. This serotonergic subsensitivity is most commonly observed as a blunted hormonal response to serotonergic drugs, such as serotonin reuptake inhibitors, in depressed individuals [e.g. (16,65)]. However, a recent review article has indicated that the findings with directly acting 5-HT receptor agonists are much less consistent with respect to hormonal subsensitivity, and has suggested that the apparent subsensitivity can be explained by a reduction in the release of 5-HT, rather than any change in 5-HT receptors (9). At present, there are no data on hormone levels in the FSL and HDS rats after challenges with serotonergic agents, so it is not possible to indicate how closely these animal models resemble depressed individuals with respect to hormonal subsensitivity. Similarly, as indicated above, there is no information on the effects of cholinergic and serotonergic drugs on temperature regulation in humans. Clearly, further studies must be performed before the serotonergic/cholinergic balance hypothesis can be accepted.

ACKNOWLEDGEMENTS

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FAILURE OF PYRIDOSTIGMINE PRETREATMENT TO PROTECT AGAINST THE EFFECTS OF CHLORPYRIFOS

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Soldiers who were deployed to the Persian Gulf War were commonly given pyridostigmine, a peripherally acting anticholinesterase (anti-ChE) agent, to protect against exposure to nerve agents, which are centrally acting anti-ChEs. The present project was designed to test this strategy in rat strains which are known to be differentially sensitive to anti-ChEs and other cholinergic agents. Both sexes of the Flinders Sensitive Line (FSL) rats, which are more sensitive to cholinergic agents, their selectively bred counterparts, the Flinders Resistant Line (FRL) rats, and randomly bred Sprague-Dawley (SD) rats were first challenged with oxotremorine (0.2. mg/kg), a centrally acting cholinergic agonist, to confirm gender and strain differences. Oxotremorine-induced hypothermia was influenced by both gender and strain: the female rats exhibited a greater degree of hypothermia than their male counterparts, and the FSL rats exhibited a greater degree of hypothermia than their FRL counterparts, with the SD rats having intermediate scores. Within 24 hours of the oxotremorine challenge, the rats began being treated chronically with pyridostigmine (12 mg/kg by gavage) or saline vehicle for 14 days. On the day of the last treatment the rats received an acute treatment of chlorpyrifos (CPF, 60 mg/kg by gavage 30 min after last chronic treatment). Body temperature and activity were recorded telemetrically via previously implanted transmitters. CPF had both strain- and gender-dependent effects and these effects interacted with pyridostigmine pretreatment. Female rats were more sensitive to the hypothermic effects of CPF than their male counterparts, as were the FSL rats compared to the FRL rats. Pyridostigmine did not alter the effects of CPF in the more resistant male rats, but potentiated its effects in the female rats. Group differences for the activity measures were not as pronounced. Thus, the more sensitive female rats, instead of being protected by the pyridostigmine pretreatment, were more sensitive to CPF. These findings provide no support for the strategy of pretreating individuals with pyridostigmine as a protection against nerve gas exposure and suggest that the strategy may make certain individuals more sensitive to these agents.

KEY WORDS Pyridostigmine, Flinders Rats, Chlorpyrifos

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SUPERSENSITIVE GROWTH HORMONE RESPONSES FOLLOWING PYRIDOSTIGMINE CHALLENGE IN A GENETIC ANIMAL MODEL OF DEPRESSION. D.H. Overstreet*, G.A. Mason, Y. Yang and A.H. Rezvani. Dept. Psychiatry, Univ. No. Carolina, Chapel Hill, NC 27599-7178.

Pyridostigmine is an anticholinesterase agent that has been widely used to study cholinergically induced growth hormone responses in humans and has also been widely prescribed as a prophylactic in soldiers deployed in the Persian Gulf War. One of the subgroups of humans who exhibit exaggerated growth hormone responses to pyridostigmine are individuals with major depression. It was predicted, therefore, that the Flinders Sensitive Line (FSL) rat, a genetic animal model of depression achieved by selective breeding for increased cholinergic responses, would exhibit greater growth hormone responses following a challenge with pyridostimine than the control Flinders Resistant Line (FRL) rat. Both sexes of the FSL, FRL and randomly bred Sprague-Dawley (SD, Harlan) rats were given either saline vehicle or 4, 12, or 36 mg/kg pyridostigmine by gavage and sacrificed by decapitation 30 min later. Telemetrically monitored temperature and activity measures, which did not differ, were reported at a previous meeting. The serum was analyzed for growth hormone using a kit from the NIDDK. Pyridostigmine produced in all groups an elevation of growth hormone that followed an inverted U-shape function, with highest levels being produced by the 12 mg/kg dose. The FSL and SD rats exhibited higher levels of growth hormone than the FRL rats at the doses of 4 and 12 mg/kg. Thus, these data confirm the cholinergic supersensitivity of the FSL rats using an endocrine challenge procedure which has reported a similar exaggerated response in depressed individuals. Supported by a contract from the U.S. Army.

Key Words: (see instructions p. 4)

1. Cholinergic Supersensitive
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